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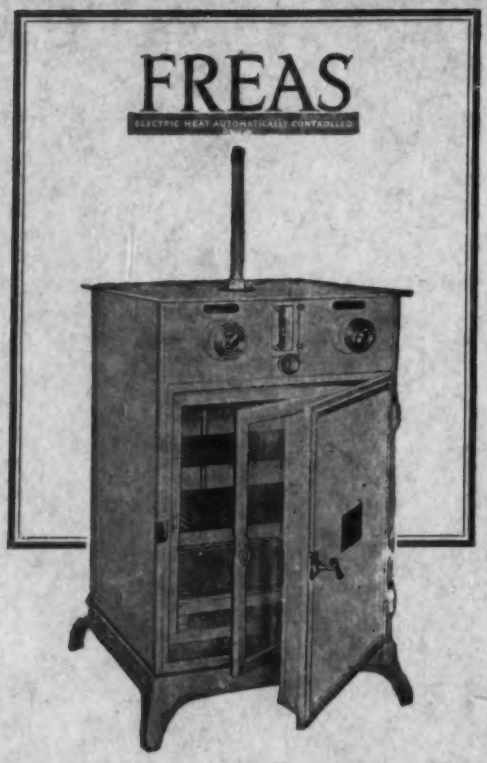

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CLINICAL AND EXPERIMENTAL

LATENT TOLERANCE IN DIABETES MELLITUS. A STUDY OF THE EFFECT OF HIGH SUGAR DIETS WITH INSULIN ON CONTROLLED DIABETICS*

BY R. B. GIBSON, PH.D., IOWA CITY, IOWA

THE administration of glucose by mouth as in the ordinary blood-sugar curve test results usually in blood-sugar values at the end of two hours which are lower than the fasting level. Autogenous hypoglycemic symptoms may be provoked following sugar ingestion.[1] When the double blood-sugar curve test of Hamman and Hirschman[2] is done on the diabetic, an increase in sugar tolerance during the second period as compared with the first is observed, as for the normal subject. Our surgical diabetics who are given insulin and sugar (orange juice and lactose) as preoperative preparation[3] withstand surgical procedures as well as or better than nondiabetic patients; when the operation is of minor character, some immediate improvement in tolerance often may be observed. Therefore, we were not unduly surprised when a patient with failing heart and edema, nephritis, and a diabetes mellitus (controlled) was thrown into a hypoglycemic reaction on the third day of our high sugar cardiac diet[4] with what we considered a safe insulin dosage for the carbohydrate given.

Accordingly, it seemed to the writer that the stimulating effect of sugar ingestion on the carbohydrate tolerance might be used in diabetic management. The results have been successful beyond expectation.

Diabetic patients admitted to our service are hospitalized and placed on a maintenance diet immediately. Protein, carbohydrate and fat are given in a proportion of 1:1:2.5 by weight with a fatty acid-glucose ratio of 1.5. The

*From the Laboratory of Pathological Chemistry, the University Hospital, the State University of Iowa.

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energy value of the diet is increased by added fat at the time of discharge, bringing the fatty acid-glucose ratio to 2.0. When indicated, insulin is given in sufficient dosage to promptly desugarize the patient without change in diet. No attention is paid to ketone excretion, unless acetone and diacetic acid are present in excess. Some subsequent improvement in tolerance may occur over a period of weeks.

When the above management is interrupted by short periods (two to three days) of high sugar ingestion, with increased insulin controlled by frequent blood-sugar determinations, a remarkable improvement in tolerance develops permitting a reduction in unit dosage when the regular diabetic diet is resumed. Successive periods of high sugar ingestion at intervals of four or five days may result each time in further improvement. It is possible to progress in this way until insulin therapy may be entirely withdrawn in some cases, even in young individuals. Two or three high sugar periods suffice.

With two mild diabetics, the high sugar period was not interrupted, but the insulin dosage was progressively reduced until discontinued on the eighth day. Substitutions and additions were then made to the high sugar diet. One of these patients, who is employed in the hospital, now on a general diet, has had normal blood-sugar values since the experiment (six months): his diabetes had been controlled for seven months before the high sugar period was started.

The first case report is given because it demonstrates so well the improvement in tolerance resulting when a high sugar period is introduced into the established management. Though marked progress is confined to the first two or three days, this period was continued into the fifth day. It seemed possible with this patient that the tolerance would improve so much that she could carry the high sugar diet with a potential glucose value of 240 gm. on the same insulin dosage required with the diabetic diet glucose value of 100 gm. This actually happened; it is a striking demonstration of the effect of the high sugar diet. With resumption of the diabetic diet, the usual reduction of the insulin is shown.

TABLE I

HIGH SUGAR DIET

Protein 46 gm., fat 117 gm., carbohydrate 205 gm.
Calories 2117; potential glucose 243 gm.

Breakfast, 7 A.M.
Milk 100, cream 66, cooked cereal 120 (20 dry oatmeal), sucrose 10, orange juice 140.

Dinner, 12 noon
Cream 132, butter 10, milk 100, cream 66, ice cream 100, candy 10, lettuce 50, orange 100, glucose 10.

Tea, 3 P.M.
Milk 100, cream 66.

Supper, 5 P.M.
Candy 20, milk 250, butter 10, aspic 100, celery 50, egg (one) 65, orange 100, glucose 20.

Lunch, 8 to 9 P.M.
Orange 150 or apple 100.

A smaller diet with calories 1978 and potential glucose of 230 gm. is also used.

Mrs. M. F., aged fifty-one, was admitted to the University Hospital Nov. 29, 1927 with a history of diabetes of eleven years standing and with a gangrenous ulcer of the right foot from a primary infection of the toes extending over the entire foot and into the lower part of the leg. She was placed on the usual diabetic management with insulin. Incisions for drainage and removal of necrotic tissue failed to induce improvement, and the right foot including the lower third of the leg was amputated December 16. At the beginning of the first sugar period, January 10, the stump had healed except for one small area which was necrotic. The patient was up and went about in a wheel chair. Her urine was free from sugar, blood sugar only slightly above normal; an equilibrium between her diet, insulin dosage, and tolerance had been maintained since December 20. Her diet consisted of protein 55 gm., carbohydrate 55 gm., and fat 137 gm., with an energy value of 1730 calories and a potential glucose of 100 gm.; insulin dosage was 46 units. Blood sugar on January 6 was 133 mg. two hours after breakfast. The high sugar period was started January 10, with 73 units of insulin this day. Her tolerance promptly increased so that on January 13 and 14 the insulin had been reduced to the presugar period figure of 46 units with a blood sugar figure on the fourteenth of 149 mg., though the glucose value of the high sugar diet was 240 gm. The patient was then returned to the regular diabetic diet with her insulin dosage 30 units (January 16). A second high sugar period of two days duration has permitted a further reduction of insulin to 24 units daily.

STATE UNIVERSITY OF IOWA HOSPITAL DIABETIC CHART

Name: Mrs. M. F., Age 51, Service Surgical, Clinical No. B 10432, Complications Arteriosclerotic gangrene of right foot. Amputation 12/16/1927.

NO.	DATE	TOTAL URINE	SP. GR.	ACETONE	DIACETIC	SUGAR %	SUGAR TOTAL	BLOOD SUGAR	INSULIN UNITS	DIET NO.	CAL.	WT. KILO.	REMARKS
1	1/6	1620	1.010	–	–	–	–	133	46	55	1730		Un. 20-11-15
2	7	825	1.012	–	–	–	–		46	55	1730		
3	8	850	1.018	–	–	–	–		46	55	1730		
4	9	1100	1.010	–	–	–	–		46	55	1730		Un. 35-18-20
5	10	1400	1.005	–	–	–	–	137	73	H. S.	2113		Un. 25-15-18
6	11	1800	1.009	–	–	–	–	168	58	H. S.	2113		Un. 22-12-17
7	12	1200	1.010	–	–	–	–	155	51	H. S.	2113		Un. 20-11-15
8	13	1025	1.010	–	–	–	–	175	46	H. S.	2113		
9	14	1300	1.010	–	–	–	–	149	46	H. S.	2113		
10	15		No	speci	men			123	36	55	1730		Un. 15- 9-12
11	16	1450	1.016	–	–	–	–	158	30	55	1730		Un. 13- 7-10
12	17							155	30	55	1730		

Case reports for Mr. C. G. and for a recently studied patient, Mr. D. R., with the charted data, illustrate the procedure which at this time seems best adapted to improve the tolerance when alternating periods of routine management and high sugar dietary are employed. These patients are young and were regarded as severe diabetics when admitted to our service. Ordinarily they would have been discharged on the insulin dosage given just prior to the time the first high sugar periods were started.

Mr. C. G., aged twenty-five, was admitted to the University Hospital Jan. 18, 1928 with a history of diabetes mellitus beginning in May, 1926 and controlled by diet and insulin by his home physician in October, 1926. He complained of pain in the lower abdomen after meals; this disappeared on the third day of our routine management and the gastric analysis and series were negative. His maintenance diet consisted of protein 55 gm., carbohydrate 55 gm., and fat 137 gm., with an energy value of 1730 calories. A satisfactory insulin dosage, 60 units daily, was established January 24. During his stay in the hospital, he was given four periods of high sugar diet of two days each; the third period was interrupted by a slight complication and is not given in the accompanying chart. Insulin dosage was reduced from 60 to 27 units a day after the first high sugar period, to

17 units after the second, and finally to 12 units after the fourth period. He was discharged on February 26 with an increase in his diet of 55 gm. of fat, bringing the calorie value to 2240. He has reported under date of April 13 that he is doing well, has gained 7 kg. in weight, and that his urine has been free from sugar.

STATE UNIVERSITY OF IOWA HOSPITAL DIABETIC CHART

Name: C. G., Weight 51, Age 25, Service Dr. B., Clinical No. C 394, Height 168

NO.	DATE	TOTAL URINE	SP. GR.	ACETONE	DIACETIC	SUGAR %	SUGAR TOTAL	BLOOD SUGAR	INSULIN UNITS	DIET NO.	CAL.	WT. KILO.	REMARKS
1	1/30	1500	1.010	–	–	–	–	138	60	55	1730	56.0	U. 25, 15, 20
2	1/31	2000	1.012	–	–	–	–	122	60	55	1730	57.9	ditto
3	2/ 1	3000	1.010	–	–	–	–	213	71	H. S.	1978	59.1	U. 33, 18, 20
4	2/ 2	2300	1.010	–	–	–	–	190	60	H. S.	1978	56.4	U. 25, 15, 20
5	2/ 3	2000	1.012	–	–	–	–	133	40	55	1730	54.6	U. 13, 7, 20*
6	2/ 4	1900	1.016	–	–	–	–	123	28	55	1730	54.6	U. 13, 6, 9
7	2/ 5	1850	1.016	–	–	–	–	129	27	55	1730	54.5	U. 12, 6, 9
8	2/ 6	1300	1.018	–	–	–	–		27	55	1730	53.2	ditto
9	2/ 7	2000	1.010	–	–	tr.	tr.	190	60	H. S.	1978	53.2	U. 25, 15, 20
10	2/ 8	1500	1.010	–	–	–	–	182	50	H. S.	1978		U. 20, 12, 18
11	2/ 9	1050	1.018	–	–	–	–	172	18	55	1730	55.5	U. 8, 0, 10
12	2/10	1150	1.018	–	–	–	–	129	17	55	1730	55.5	U. 10, 0, 7
13	2/11	1600	1.012	–	–	–	–	140	17	55	1730		ditto
14	2/12	1000	1.022	–	–	–	–	122	17	55	1730	54.1	ditto
15				–	–	–	–						
16	2/19	1150	1.026	–	–	–	–	128	16	55	1730		U. 10, 0, 6
17	2/20	900	1.020	–	–	–	–	149	14	55	1730	53.9	U. 8, 0, 6
18	2/21	1500	1.012	–	–	–	–	133	55	H. S.	1978		U. 25, 12, 18
19	2/22	1600	1.010	–	–	–	–	150	42	H. S.	1978	54.0	U. 18, 10, 14
20	2/23	925	1.018	–	–	–	–	145	12	55	1730	54.0	U. 7, 0, 5
21	2/24	1600	1.016	–	–	–	–	155	12	55	1730		U. ditto
22	2/25	1050	1.024				–	149	12	55	1730	53.9	ditto
23	2/26			Discharged				140	12	55F	2241		ditto

*Given by error; added orange juice to supper.

Mr. D. R., aged eighteen, was admitted to the University Hospital May 15, 1928 for diabetes mellitus. Sugar had been found in the urine early in January of this year during an attack of mumps, though a nocturia had been noted two weeks earlier. Home treatment was dietary only, and he had lost 13.5 kg. when he entered our service. Blood sugar at 5 o'clock on the afternoon of admission was 350 mg. and the urine contained much

STATE UNIVERSITY OF IOWA HOSPITAL DIABETIC CHART

Name: Mr. D. R., Weight 43.4, Age 18, Service Dr. B., Clinical No. C. 3161, Height, 165.

NO.	DATE	TOTAL URINE	SP. GR.	ACETONE	DIACETIC	SUGAR %	SUGAR TOTAL	BLOOD SUGAR	INSULIN UNITS	DIET NO.	CAL.	KILO. WT.	REMARKS
1	6/ 5	1800	1.010	–	–	–	–	122	30	55	1730	43.9	U. 20, 0, 10
2	6/ 6	2600	1.008	–	–	–	–	155	69	H.S.	1978	43.9	30, 17, 22
3	6/ 7	1750	1.010	–	–	tr	tr	234	57	H.S.	1978	43.9	22, 15, 17
4	6/ 8	1475	1.010	–	–	–	–	105	19	55	1730		12, 0, 7
5	6/ 9	1150	1.014	–	–	–	–	100	17	55	1730	44.1	10, 0, 7
6	6/10	1350	1.014	–	–	–	–	127	14	55	1730		8, 0, 6
7	6/11	1075	1.014	–	–	–	–	130	14	55	1730	43.2	
8	6/12	1400	1.012	–	–	–	–	96	14	55	1730		
9	6/13	2200	1.010	–	–	–	–	186	60	H.S.	1978		25, 15, 20
10	6/14	2050	1.010	–	–	–	–	138	45	H.S.	1978	43.6	20, 10, 15
11	6/15	1600	1.012	–	–	–	–	198	5	55	1730	43.6	5, 0, 0
12	6/16	1450	1.014	–	–	–	–	172		55	1730		
13	6/17	1300	1.012	–	–	–	–	168		55	1730		
14	6/18	1300	1.012	–	–	–	–	162		55	1730		
15	6/19	900	1.020	–	–	–	–	136		55	1730	42.7	
16	6/20	1300	1.012	–	–	–	–			55	1730	43.4	
17	6/21	1200	1.014	–	–	–	–			55	1730		
18	6/22	1350	1.012	–	–	–	–	149		55	1730	43.2	
19	6/26	1400	1.010	–	–	–	–	132		55	1730	43.2	

sugar. He was placed on a diet of protein 55 gm., carbohydrate 55 gm., and fat 137 gm., with an energy equivalent of 1730 calories, and was given insulin the following day. The glycosuria was controlled on May 23 with 52 units of insulin this day. Insulin was reduced gradually to 30 units on June 3, the blood sugar on June 5 being 122 mg. against a blood sugar of 107 mg. on the previous dosage of 37 units. The first period of the high sugar diet with increased insulin on June 6 and 7 permitted a reduction of the previous 30 units to 14 units daily. Insulin was discontinued after the second high sugar period, June 13 and 14; blood sugar was 198 mg. on returning to the diabetic diet, but fell to 136 mg. on June 19 and 132 mg. on June 26.

The following summaries are from the records of two mild diabetics one of whom (H. P. W.) had been controlled for six months and the other (D. O'B.) for a year previous to the experiment. Both were given the high sugar diet and this was not interrupted. Insulin was reduced daily until discontinued on the seventh and sixth days respectively. Blood-sugar tolerance tests on H. P. W. were done before and after the completion of the insulin course (Table IV). Substitutions and additions were made to this patient's diet until the high carbohydrate follow-up diet (Table II) was attained. His improvement has been maintained and he is now after six months on a general diet. The second patient, D. O'B., was transferred after a day without insulin directly to a high carbohydrate follow-up diet. Except for the second day of this diet (high carbohydrate), his blood sugar remained normal and he was discharged after a week without being returned to his previous diabetic diet.

Mr. H. P. W., aged twenty-six, weight 77 kg., a medical student, found sugar in his urine in the spring of 1927. As he was employed for part time in this hospital, he was put on a diet prepared in our therapeutic diet kitchen and kept under observation. His diabetes, which was mild in type, was easily controlled. A blood-sugar curve tolerance test on October 27 indicated that the diabetic condition was at this time even more pronounced than a previous test in June had indicated. On November 13, he was given the high sugar diet. The insulin dosage was controlled by blood-sugar determination two hours after the three principal meals daily. Twenty-four hour urine collections were inconvenient and were not made, but qualitative tests on specimens collected from time to time were always negative for sugar. He was given 48 units of insulin (in 3 doses, 20, 12, and 16 units) the first day and this was progressively diminished until discontinued on November

TABLE II

FOLLOW-UP DIETS (H. P. W. AND D. O'B.)

Protein 89 gm., fat 149 gm., carbohydrate 233 gm.
Calories 2705; potential glucose 300 gm.

Breakfast
Milk 100, cream 100, bread 30, butter 10, two eggs, cereal (dry) 20, orange 100, sugar 20.

Dinner and supper
Milk 100, cream 100, bread 30, butter 20, meat 75, potato 100, 5 per cent vegetables 100, 10 per cent vegetables 100, 10 per cent fruit, 100.
Protein 81 gm., fat 121 gm., carbohydrate 196 gm.
Calories 2209; potential glucose 254.5 gm.

Breakfast
Milk 100, cream 50, bread 30, butter 20, two eggs, cereal (dry) 20, orange 200.

Dinner and supper
Milk 100, cream 50, bread 30, butter 15, meat 75, potato 75, 5 per cent vegetables 100, 10 per cent vegetables 100, 10 per cent fruit 150.

21, the eighth day of the diet. A blood sugar curve tolerance test on November 24 showed a marked improvement over the result on October 27. Blood sugar values during the insulin period varied from 100 to 155 mg., and were from 100 to 128 mg. after the insulin was discontinued and the diet given a more general character.

TABLE III

EFFECT OF A HIGH SUGAR DIET WITH INSULIN ADMINISTRATION ON A MILD DIABETIC (H. P. W.)

DATE	INSULIN	BLOOD SUGAR 9:30 A.M.	2:30 P.M.	7:30 P.M.	DIET
11/13	0 0 0	123			Diabetic diet
14	20 12 16	117	129	103	High sugar
15	15 9 10	122	112	108	
16	11 7 8	133	120	106	
17	9 5 7	145	122	103	
18	9 0 7	130	136	100	
19	7 0 0	122	155	122	
20	5 0 0	103	137	137	
21	0 0 0	125	122	122	
22	0 0 0	122			
23	0 0 0	125			
12/ 1		109			Diet more general in character, high chh. and low salt (Table II)
8		117			
15		100			
30		125			
1/ 7		120			
31		126			
3/4		128			
27		127			
5/3		127			
6/24		122			General, since 5/15

TABLE IV

GLUCOSE TOLERANCE TEST ON H. P. W. BEFORE AND AFTER THE HIGH SUGAR DIET PERIOD

HOURS	OCTOBER 27 BLOOD SUGAR MG.	URINE SUGAR	NOVEMBER 24 BLOOD SUGAR MG.	URINE SUGAR
0.0	128	none	126	none
		Glucose 50 gm.		
0.5	220		179	
1.0	235	trace	179	none
1.5	197		143	
2.0	165	trace	127	none
2.5	122		120	
3.0	109	trace	111	none

Mr. D. O'B., aged fifty-nine, was admitted to the University Hospital March 3, 1928, for diabetes mellitus. He had been managed successfully during a previous admission (May 10, 1927 to June 1, 1927) with diet alone. The diabetes was mild and had not progressed. Examination revealed only a slight secondary anemia. There was a gastric resection, which had been done two years previously, with a well functioning gastrointestinal opening. He was placed on a diabetic diet, then given two days of general diet to demonstrate the diabetic condition, after which he was returned to dietary management. The high sugar diet was started on March 14, with 44 units of insulin, and was continued with daily insulin reductions as in the case of H. P. W. Insulin was discontinued on March 20, when the blood sugar was 100 mg. He was then given a high carbohydrate diet of general character and was discharged a week later with a final blood sugar 110 mg.

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Name: D. O'B. Weight 59.9, Age 59, Service Dr. F. M. S., Clinical No. B 4251 (Ad. 2), Height 168

NO.	DATE	TOTAL URINE	SP. GR.	ACETONE	DIACETIC	SUGAR %	SUGAR TOTAL	BLOOD SUGAR	INSULIN UNITS	DIET NO.	CAL.	KILO. WT.	REMARKS
1	3/ 6	700	1.024	–	–	–	–	108		55	1730	59.9	
2	3/ 7	700	1.024	–	–	–	–			55	1730		
3	3/ 8	300	1.024	–	–	–	–			55	1730	58.7	
4	3/ 9	725	1.030	tr.	–	tr.	tr.	290		Gen.			
5	3/10	700	1.020	tr.	–	tr.	tr.			Gen.		58.4	
6	3/11	900	1.020	–	–	–	–			55	1730	59.1	
7	3/12	900	1.012	–	–	–	–			55	1730		
8	3/13	1100	1.020	–	–	–	–	120		55	1730		
9	3/14	950	1.020	–	–	–	–	84	44	H.S.	1978		U. 22, 10, 12
10	3/15	675	1.018	–	–	–	–	120	35	H.S.	1978		U. 15, 8, 12
11	3/16	1400	1.010	–	–	–	–	119	26	H.S.	1978		U. 12, 6, 8
12	3/17	750	1.018	–	–	–	–	105	16	H.S.	1978	58.6	U. 9, 0, 7
13	3/18	1450	1.012	–	–	–	–	77	12	H.S.	1978		U. 7, 0, 5
14	3/19	900	1.010	–	–	–	–	106	5	H.S.	1978		U. 5, 0, 0
15	3/20	1000	1.010	–	–	–	–	100		H.S.	1978		
16	3/21	950	1.020	–	–	–	–	108		H.C.	2208		
17	3/22	1350	1.012	–	–	–	–	149		H.C.	2208		
18	3/23	1100	1.014	–	–	–	–	104		H.C.	2208		
19	3/24	1200	1.020	–	–	–	–			H.C.	2208	60.0	
20	3/25	1450	1.014	–	–	–	–	103		H.C.	2208		
21	3/26	1800	1.014	–	–	–	–			H.C.	2208		
22	3/27	1100	1.020		–	–	–	110		H.C.	2208	59.9	

Of 19 patients who have received high sugar diets, but 3 have failed to react favorably. One of these was a case of fracture of the leg with delayed healing, the second a twelve-year-old girl with an unstable response to usual management, and the third a middle aged woman to whom the high sugar diet was given without previous control. The experiment was discontinued on one patient who failed to cooperate, and on a second who developed an intestinal complication; both had shown the usual improvement in tolerance after high sugar periods.

Patients whose courses can be followed and who are assured of adequate home management after leaving the hospital are now being selected for the high sugar dietary management. It is important to know how permanent the improved tolerance is and what changes in the diet may prove more efficient. Again, what further increase in tolerance, if any, is possible for return cases previously given the high sugar diet?

It is difficult to correlate the results with current work in diabetic management and the action of insulin. Sansum, Blatherwick and Bowden's[5] diabetic control and Bainbridge's[6] study on rats both indicate that insulin is much more effective when a diet high in carbohydrates is employed as compared with a high fat ration. Macleod[7] has suggested a stimulation of insulin production by glucose acting on the islet cells not yet involved in the disease process. Chabanier and Copeman[8] have attempted to treat diabetes with relatively high carbohydrate diets and large doses of insulin; when the patient has been desugarized for fifteen days and blood sugar normal, the injections may be suspended pending a return of further symptoms, when another "series" is undertaken. The free intervals between series were found to increase gradually with progressively diminished insulin

periods. Sweeny's[9] recent experiments on the effect of diet on sugar tolerance are of interest in showing that diets high in fat and protein and low in carbohydrate result in lowered sugar tolerance. Latent tolerance may be due to a fixation of the sugar mechanism at a low level unless stimulated by sugar ingestion, a condition which is exaggerated in diabetes and continued, though at a different level, in the controlled diabetic. The idea that a dormant rather than a functionally inadequate mechanism may obtain in diabetes and that it may be stimulated to activity under appropriate conditions suggests itself. This is in accord with the fact that in a considerable proportion of cases no pathologic changes can be found in the pancreas (Adami and McCrae).

SUMMARY

Diabetics controlled on maintenance diets with a fatty acid, potential glucose ratio of 1.5 and the required insulin dosage have shown a remarkable improvement in tolerance following two or three days of high sugar ingestion with increased insulin. Successive periods of high sugar diet at intervals of four or five days result usually in a progressive increase in tolerance until dietary management without insulin may suffice to control the diabetes, especially in young individuals. Two mild diabetics, previously controlled, continued to improve in tolerance without interruption of the high sugar diet except for substitutions and additions until a diet of general character was attained.

The insulin used in this study was in part supplied through the courtesy of E. R. Squibb & Sons.

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COLLOIDAL IRON, ELIMINATION THROUGH THE GASTROINTESTINAL CANAL*

By M. H. Streicher, M.D., Chicago

INTRODUCTION

A REVIEW of the literature on internal iron metabolism brings out many questions still open to discussion. Many investigations have been made on the chemistry of iron metabolism, yet no confirmation has ever been received through morphologic researches of the intestinal canal with especial reference to studies of colloidal iron.

There is still some controversy as to whether iron when given intravenously in large doses and over a long period of time is excreted with the bile, resorbed by the duodenum and later excreted in the large intestine, or whether it is resorbed by the cecum before it is eliminated in the stool.

Cushny states that iron injected intravenously into animals is stored in the liver, spleen and bone-marrow, but is taken up from these organs again and is excreted by the epithelium of the cecum and colon. We were primarily interested in determining the cells that have a special affinity for colloidal iron substances in the course of elimination through the intestine.

LITERATURE

In a careful résumé of the literature it is disappointing to note the degree of variance among different investigators as to structural components chiefly concerned in the elimination of iron and distribution of iron pigment.

Stockmann and Greig (1897) state that iron is excreted from the blood chiefly by the intestinal mucous membrane, although minimal amounts are present in the bile and urine.

Beattie (1905) in his report on Hemochromatosis states that he has not found any pigment containing free iron in any part of the gastrointestinal tract.

Muir and Dunn (1914) report a positive naked-eye test for iron in the mucous and muscular coats of the stomach and a negative test in the colon. Microscopically they found few iron granules in the mucous and submucous coats of the small intestine.

Austin and Pearce (1914) state that the elimination of iron occurs almost entirely through the intestine especially the colon.

Dillard and Weidman (1925) in their report of a necropsy of a Multiple Hemorrhagic Sarcoma of Kaposi state that the colon demonstrated clusters of pigmented endothelial cells in the mucous stroma, but no pigment was found in the small intestine, suggesting some eliminative process in connection with this pigment.

*From the Department of Pathology and Medicine, University of Illinois, College of Medicine and the Grant Hospital.

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TABLE I

ELIMINATION OF COLLOIDAL IRON, SHOWING RELATIVE DISTRIBUTION OF IRON IN THE INTESTINAL CANAL

DIVISION OF THE GASTRO-INTESTINAL CANAL	RABBIT NO. 4 20 INJECTIONS			RABBIT NO. 5 20 INJECTIONS			RABBIT NO. 6 60 INJECTIONS			RABBIT NO. 12 30 INJECTIONS			CONTROL, NO INJECTIONS RABBIT NO. 7		
	EP.	ST.	L.T.	EP.	ST.	L.T.	EP.	ST.	L.T.	EP.	ST.	L.T.	EP.	ST.	L.T.
Stomach	0	+	0	0	+	0	0	+	0	0	+	0	0	0	0
Duodenum	0	0	0	0	+	0	0	0	0	0	+	0	0	0	0
Jejunum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ileum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sacc. Rotundus	0	0	+	0	0	+	0	0	+	0	0	0	0	0	0
Appendix	0	0	+	0	0	+	0	0	+	0	0	+	0	0	0
Cecum	3+	+	0	4+	+	0	60+	+	0	6+	6+	2+	0	0	0
Colon	0	+	0	0	+	0	0	+	0	0	0	0	0	0	0

Ep.—Epithelium.
St.—Stroma.
L.T.—Lymphoid tissue.

Pfeiffer and Boerner Patzelt claim that very few iron cells are found in the stroma of the villi of the duodenum. Many iron containing cells are found in the stroma of the mucosa of the cecum. Few iron containing cells are seen in the stroma of the mucosa of the colon. Masses of iron granules are found in the cells of the reticuloendothelial system.

MATERIALS AND METHODS

The data of the present investigation are based on experiments on rabbits using intravenous injections of a colloidal iron preparation. Daily injections of 2 c.c. of a 20 per cent solution of saccharated iron oxide were given for a period of twenty to sixty days.

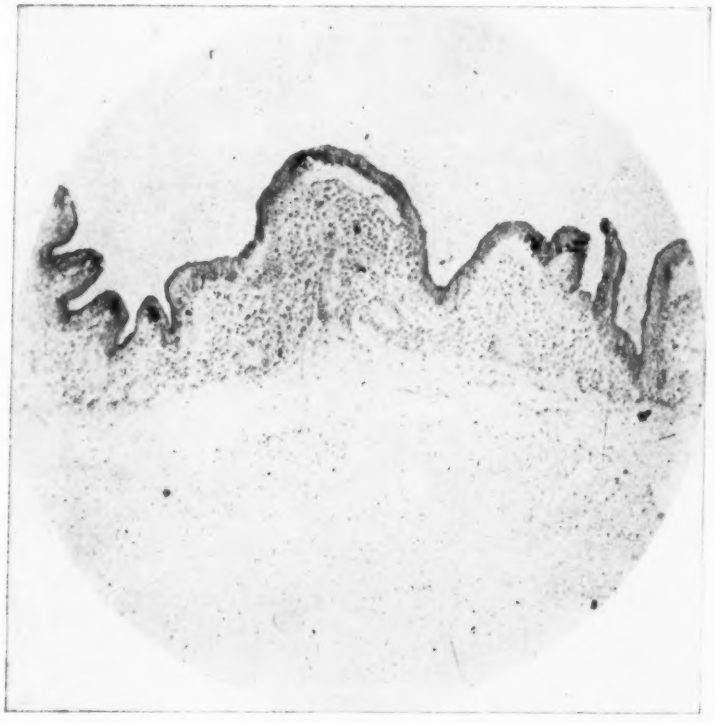

Fig. 1.

The animals were killed by bleeding, and necropsied. Sections were taken from different portions of the gastrointestinal canal, tissues fixed in 94 per cent alcohol, embedded in paraffin, and stained after the Prussian blue method for iron pigment, lithium carmin being used as counterstain.

The naked-eye test (Pearl-Prussian blue method) was also applied in each case before fixation of tissues. The bile was collected and tested for relative amount of iron present.

RESULTS

Saccharated iron oxide has the same action on body tissues as vital stains have, that is, iron granules are stored by the same cells that take up such dyes as lithium carmin, trypan blue, etc.

In using vital stains the intensity of stain will necessarily depend upon its concentration, and therefore, we would expect to find maximum concentration in that portion of the intestinal canal where elimination is most pronounced. Table I shows the relative distribution of iron pigment in the epithelium, stroma and lymphoid tissue.

Table I demonstrates distinctly that of the intestinal epithelium, that of the cecum is chiefly concerned in the process of elimination of iron introduced in colloidal form. No pigmentation was found in the duodenum and colon. Apparently only a small amount of iron leaves the body through the bile.

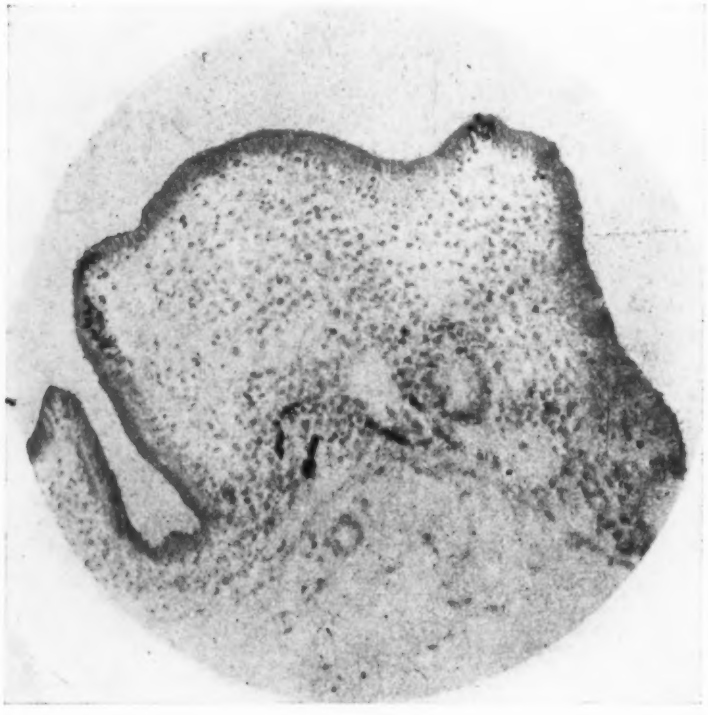

Fig. 2.

The histologic analysis of the cecum may be best demonstrated by a representative photomicrograph. Fig. 1 is a photomicrograph of the cecum under low power in which the general distribution of the iron granules is shown by deposits of black particles along the lining epithelium of the cecum.

Fig. 2 is a photomicrograph of a portion of a cecum under high power in which the granules are seen to be deposited in the lining epithelium and also in the histocytes of the stroma.

DISCUSSION

It is a generally accepted view that certain cells will become vitally stained when colloidal metallic substances are injected into the blood stream. Eppinger was the first to show that the reticuloendothelium possessed the

property of storing colloidal iron pigment. In considering the cellular elements that are specifically concerned in this process, we refer to:

1. The cells of the reticuloendothelial system as in the liver, spleen, lymph glands and bone-marrow.

2. The liver cells and the epithelial cells of the convoluted tubules of the kidney when numerous injections are given.

3. The epithelium of the cecum and the histocytes in various portions of the intestinal canal.

Some investigators claim that iron when given by mouth, is first absorbed by the duodenum and later excreted in the large intestine especially by the cecum. Yet, others believe that iron stored in the liver is excreted chiefly by the bile and is resorbed to some extent in the large intestine.

If it is true that the iron stored in the liver is excreted in the bile, then we should expect an increase of the iron content in the bile; or if it is true that absorption is taking place in the duodenum, then we should find iron granules stored in the epithelium of the mucosa; but the iron content of the bile is not increased in these experiments. This is supported by Cushny who states "that iron stored in the liver does not escape by the bile." Although a small percentage of iron is a constant constituent of this fluid, it is not increased when iron is given intravenously. This view is supported by Dubin and Pearce (1918), who state that in a dog, iron in appreciable amounts is not eliminated in the bile. Again, iron granules were not found to be stored in the mucosa of the duodenum. Hence, we conclude that colloidal iron given intravenously is not eliminated in the bile in large amounts and that apparently no absorption takes place in the duodenum.

CONCLUSION

Colloidal iron given intravenously in appreciable amounts is eliminated by the cecum.

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THE CLINICAL USE OF A COLLOIDAL ALUMINUM HYDROXIDE AS A GASTRIC ANTACID*

By Burrill B. Crohn, M.D., New York, N. Y.

FOR the last several years internists interested in gastroenterology have felt a certain dissatisfaction with the increasing use of soluble alkaline salts to reduce or neutralize gastric acidity (hyperacidity). Laboratory tests with the fractional test meal as a medium of experimentation have shown that soluble alkalies like bicarbonate of soda, when administered by mouth, cause an immediate and total neutralization of all the gastric acidity (with a temporary suspension of digestion). This is followed by a rapid rebound of gastric acidity resulting within a half hour in an hyperacidity of even greater degree than in a control curve. These same results hold equally well in the case of the less soluble magnesia salts, such as magnesium oxide and magnesium phosphate.

In 1917 definite deleterious toxic effects were noted by Hardt and Rivers[1] to result from excessive dosages of soluble alkalies particularly in cases of gastroduodenal ulceration. Here the prolonged administration of such drugs in large doses, frequently given over long periods of time, resulted in definite untoward clinical states characterized by headache, malaise, anorexia, nausea and vomiting, diarrhea and marked prostration. Gastric tetany, one of the most dreaded of complications particularly in ulcer cases, while probably not caused by actual over-alkalinization, is definitely closely associated with a condition of plasma alkalosis as evidenced by the high CO_2 coefficient in the plasma of such cases.

The dissatisfaction with the soluble alkalies has suggested the greater popularization and wider use of insoluble neutral salts which, dissociating in the gastric secretion, result in sufficient neutralization to give clinical relief. Such salts, containing calcium as the carbonate or phosphate of lime, are not absorbable as such from the gastrointestinal tract and are thus devoid of toxic after-effects. Greenwald[2] has also pointed out that the neutral secondary and tertiary phosphates of magnesium and calcium acted as local antacids without producing systemic alkalinization.

The introduction during the last few years of a special colloidal form of aluminum hydroxide as a gastric antacid has interested me as offering perhaps an optimum medium for the reduction of gastric acidity (and so-called hyperacidity), and as suggesting a salt which is devoid of any secondary acid-stimulating effect. It has also seemed desirable to administer only such a substance which, while reducing acidity to a point where clinical heartburn and pain are relieved, will yet not completely alkalinize the gastric chyme and so

*Colloidal aluminum hydroxide is marketed by The Wander Company of Chicago, under the trade name "Alucol."

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suspend digestive activity. With this in mind I have completed some simple comparative studies on the effects of these latter nonabsorbable and nonsoluble salts noting their action in vivo and also their clinical possibilities and possible toxic effects.

Calcium phosphate (in doses of 30 grains, 2 grams) administered to a patient with a normally high acid curve is a rather ineffectual antacid (Fig. 1). Free acid is seen in the fractional test meal to be only very moderately reduced, the total acidity remaining practically unaltered.

Calcium carbonate in similar dosage when administered toward the end of a test meal is more efficient as an antacid (Fig. 2) but causes, like the soluble sodium salts, a complete gastric neutralization.

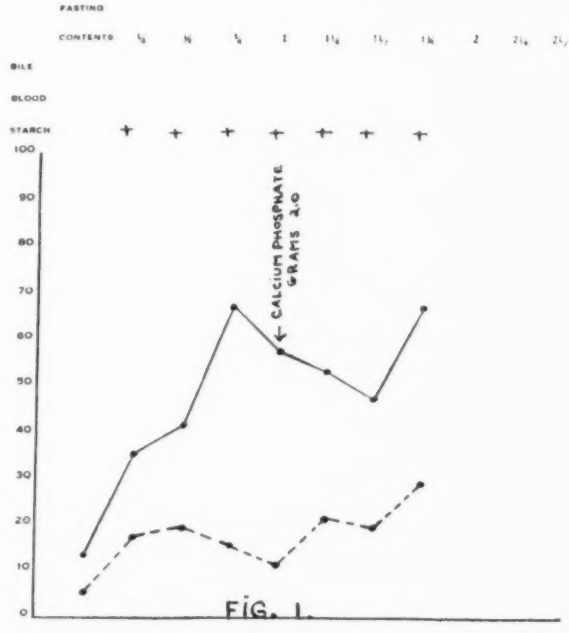

FIG. 1.

Colloidal aluminum hydroxide, representing a successful and permanent colloidal suspension of aluminum hydroxide, offers many advantages. It is a neutral salt; it cannot produce an alkaline reaction in the gastric chyme and is therefore incapable of suspending or paralyzing digestion; it is an efficient antacid which by reducing the free acidity to a minimum consistent with continued digestion, also reduces the total acidity to a point where complete cessation of subjective symptoms is almost an invariable rule; it is nonabsorbable and nontoxic. Fig. 3 represents the control curve in a case of gastric hyperacidity with subjective symptoms of heartburn and postprandial discomfort. On a successive day 30 grains of colloidal aluminum hydroxide were administered to the same patient three-quarters of an hour after the test meal had been given and when the mounting acid curve showed that the conditions were similar to the day of the former control test (Fig. 4). The effect of the colloidal aluminum hydroxide on the gastric curve is readily

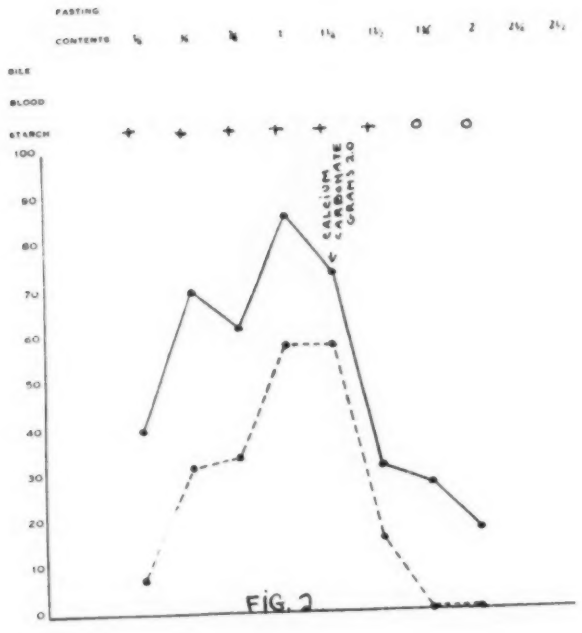
FASTING
CONTENTS
BILE
BLOOD
STARCH
CALCIUM CARBONATE GRAMS 2.0
100
90
80
70
60
50
40
30
20
10
0

FIG. 2

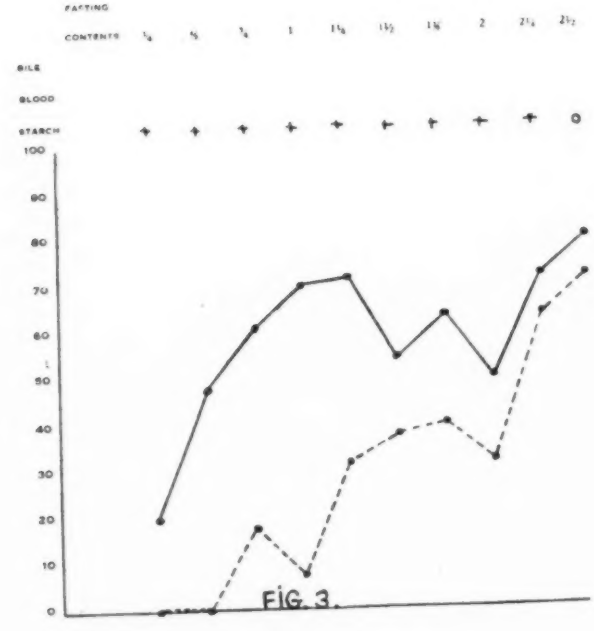
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FIG. 3.

demonstrable. Free and total acid are immediately reduced, and yet a minimum of free acidity is at all times retained allowing of continued digestion. Motility is beneficently hastened, for while in the control curve the emptying time is two and a quarter hours, after the administration of the colloidal aluminum hydroxide emptying time of the viscus is reduced to one and one-quarter hours.

Results similar to the above have been found in repeated experiments both on the same individual and on several types of cases. Deleterious or toxic effects have never been noted, and the action of the drug as an antacid in vivo is universally favorable.

Clinical Results.—I have administered colloidal aluminum hydroxide in a large series of clinical cases (fifty or more) comprising within this group

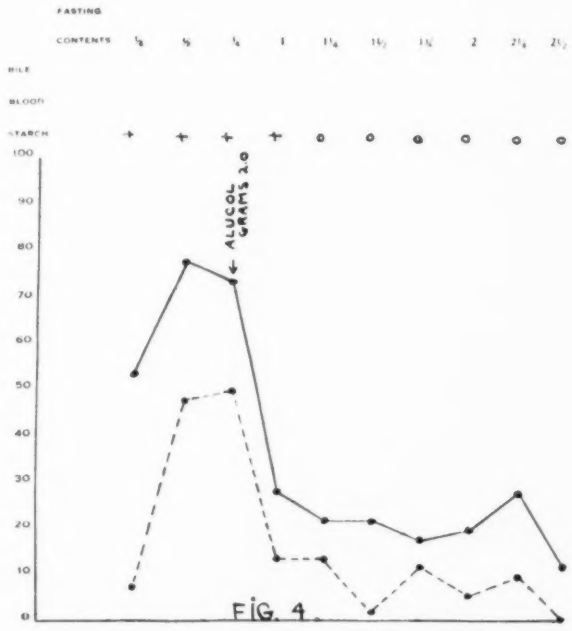

FIG. 4

examples of functional gastric secretory disturbances of the hyperacid type complaining of heartburn, belching, discomfort and constipation, cases of gastric neurosis, and also a group consisting of gastroduodenal ulcerations. In cases of functional hyperacidity and moderate subacidity with subjective complaints of pain and heartburn the relief afforded by 15 to 30 grains of colloidal aluminum hydroxide is almost immediate. The relief so afforded may last for thirty to sixty minutes only occasionally being followed by recurrence of heartburn, or the subsidence of symptoms may be complete and enduring. An undesirable constipating effect such as is seen with the bismuth salts has not been observed, nor have nausea, vomiting or diarrhea or any other toxic by-effects been noted on any occasion.

In the series of over 20 cases of gastroduodenal ulceration colloidal aluminum hydroxide serves all the purposes of an ideal antacid in giving immediate

relief to the symptoms of pain and heartburn. The pain of ulcer ceases almost immediately after the colloidal hydroxide of aluminum has been swallowed. In the less severe cases the relief endures until the next meal. In the more severe cases the relief is just as efficient and prompt, but a recurrence of the pain may not be prevented after some time. By thus giving prompt and continued relief to the symptoms of pain and heartburn it allows of a more gradual and steady increase of the diet and thus probably to more ideal conditions for a rapid healing of ulcer.

To sum up, it may be said that colloidal aluminum hydroxide seems to be the more desirable of the neutral nonabsorbable antacid salts in so far as it is an efficient agent in reducing gastric acidity to a point where symptoms are relieved but gastric digestion allowed to continue. It hastens gastric emptying; it is nontoxic and devoid of deleterious by-effects. It is clinically applicable in cases of gastric secretory disturbances characterized by hyperacidity and can be used in ulcer cases in moderate dosage over prolonged periods without the anxiety of producing or the production of alkalosis or the toxic symptoms such as may be due to the absorption of soluble alkaline salts.

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IMMUNIZATION TO SCARLET FEVER OF PROVIDENCE CITY HOSPITAL STUDENT NURSES*

By Edwin M. Knights, M.S., Providence, R. I.

IN JANUARY, 1927, the laboratory of the Providence City Hospital extended its research work on scarlet fever to include the Dick testing of student nurses and the immunization of a certain percentage of nurses showing a positive Dick test. The results here tabulated cover the period from January 1 to December 31, 1927. The work of immunizing the student nurses is being continued this year, but for reasons to be explained later the Dick testing has been discontinued and only the immunization work is being carried on.

Before the work was started in 1927 an investigation of the methods used in the production of scarlet fever streptococcus toxins and antitoxins by various commercial biologic laboratories was carried out. We were able to obtain from one of these commercial laboratories a written statement to the effect that their scarlet fever streptococcus toxins and antitoxins were produced from pooled strains of hemolytic streptococci, some of which were subcultures of the strains used by the Drs. Dick in producing experimental scarlet fever in human beings. In addition certain strains of hemolytic strepto-

*From the Laboratories of the City Hospital, Providence, R. I.
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cocci were used which were obtained from scarlet fever patients, but which had not definitely been shown to have caused scarlet fever in human beings. The products of this one commercial biologic laboratory have been used exclusively in this work.

The care of scarlet fever patients comprises part of the training of the student nurses at the Providence City Hospital, and during their stay here the students may be considered to be more or less equally exposed to scarlet fever. During the year 1927 there were 274 student nurses in training at this hospital. The period of training here may be either three or four months and there are no set dates for the arrival and departure of large groups of nurses. It might be said that students are arriving continually and leaving as regularly, having completed their period of training. Of these 274 student nurses 196 were given Dick tests for susceptibility to scarlet fever using 0.1 c.c. of standardized toxin intracutaneously, the results being read after twenty hours.

Of the 196 nurses tested there were 53 who gave a history of having previously had scarlet fever. The results of the Dick test on these 53 were 11 positive and 42 negative. There were 143 nurses who gave no previous history of scarlet fever and the results of their tests were 37 positive, 101 negative and 5 doubtful. There were in all 48 nurses or 24.5 per cent of those tested who gave a positive Dick test, and 143 or 72.9 per cent who gave a negative test, and 5 nurses or approximately 2.5 per cent who gave doubtful reactions. Twenty-six of the nurses who gave positive Dick tests and who gave no previous history of having had scarlet fever were actively immunized against scarlet fever by the method of giving five injections of scarlet fever streptococcus toxin at weekly intervals, beginning with the first injection, containing five hundred skin test doses of toxin and increasing the amount of toxin on each successive dose until the fifth and last dose contained 60,000 skin test doses. Not one of the twenty-six nurses thus immunized contracted scarlet fever during her period of training at this hospital. We have no record of their histories subsequent to the completion of their training here.

Fifteen of the 274 student nurses contracted scarlet fever during their course of training in 1927. None of these 15 nurses had been previously immunized, but Dick tests had previously been done on eight of them. Four had given positive tests, 3 had given negative tests, and one gave a doubtful test. One of the fifteen nurses contracting scarlet fever in 1927 gave a history of having had scarlet fever in 1917. Her Dick test previous to the onset of the disease had been positive and a retest made after convalescence was negative.

The twenty-six nurses who were immunized against scarlet fever were, with one exception, given a retest two weeks after their last inoculation. The results of this retest were: twenty gave negative tests, four gave positive tests, and one gave a doubtful test.

During the year we were furnished with one lot of skin-test toxin which when used in the regular way seemed to be giving too high a percentage of negative results. A fresh lot of toxin was obtained and this lot gave results at variance with the previous tests when repeated on the same nurses. The

commercial biologic laboratory which supplied us with material for this work has, during the present year, been unable to supply us with scarlet fever streptococcus toxin standardized for skin test work. In a letter to us they state, "We have shipped no Dick test for the past month. About the middle of January, all our stock was called back to the laboratory. It will be released at a time when laboratory tests prove satisfactory."

In summarizing the results obtained during the year 1927 we may draw two conclusions:

1. The Dick test toxin with which we were furnished during 1927, gave unsatisfactory results as an indicator of susceptibility to scarlet fever.

2. While the number of nurses who were given the immunizing doses of scarlet fever streptococcus toxin is rather small, the results seem encouraging enough to warrant continuing, during the coming year, the immunization of nurses who give no history of having had scarlet fever, leaving a certain percentage not immunized for controls.

DICK TESTS AND IMMUNIZATION OF NURSES, 1927

Number of nurses who served part or all of their training period in 1927	274
Number of nurses tested for susceptibility to scarlet fever	196
Number of nurses with positive Dick tests	48
Number of nurses with negative Dick tests	143
Number of nurses with doubtful Dick tests	5
Number of the 274 developing scarlet fever	15
Number of nurses developing scarlet fever with previous positive Dick test	4
Number of nurses developing scarlet fever with previous negative Dick test	3
Number of nurses developing scarlet fever with previous doubtful test	1
Number of nurses developing scarlet fever not previously tested	7
Number of nurses immunized (5 doses) (one received only 3 doses)	26
Number of nurses with positive Re-Dick after immunization	4
Number of nurses with negative Re-Dick after immunization	20
Number of nurses not tested after immunization	1
Number of nurses with doubtful test after immunization	1
None developed scarlet fever who had previously received active immunization	

Dick Tests

	POS.	NEG.	DOUBTFUL	TOTAL
Nurses with previous history of scarlet fever	11	42	0	53
Nurses with no previous history of scarlet fever	37	101	5	143

SECONDARY FACTORS IN UNCOMPLICATED CASES OF SEASONAL HAY FEVER

Based on a Study of Seven Hundred Nineteen Cases

By Ray M. Balyeat, M.A., M.D., F.A.C.P., Oklahoma City, Okla.

DURING the first seven years of our study of allergic diseases uncomplicated cases of seasonal hay fever were tested only with pollen. During the latter part of this period, however, we became suspicious, on account of failures in obtaining good results in some cases, that some seasonal hay fever patients might be hypersensitive to other atopic substances than pollen, which would act as secondary factors. For this reason cases obtaining poor results were rechecked for the possibility of sensitivity to animal dander, other dusts, and foods. During the past two years all cases of seasonal hay fever who manifested no symptoms out of the pollen season were tested both by the scratch and intradermal methods, with pollen, animal epithelial, other common inhalants, and some of the important foods, just as we routinely do cases of perennial hay fever.

Seven hundred and nineteen private uncomplicated seasonal hay fever cases which have been investigated in our Clinic during the past two years comprise our present study. Our findings relative to a sensitivity to inhalants other than pollen in these cases interested us since they had no symptoms out of season. We believe that our knowledge of the findings enhanced the value of our management very materially, which has encouraged this presentation.

POLLEN THE SOLE CAUSE

Of the 719 cases studied, 345, or 47.9 per cent, were not found sensitive by either method of testing, to animal dander, other common dusts, orris root, eggs, milk, and wheat.

POLLEN AS A SECONDARY FACTOR

One hundred and twelve cases of seasonal hay fever which had no symptoms prior to August 12 were studied, all of which showed a marked reaction to one or all of the three ragweeds. Of this group, 57, or 50.8 per cent, showed a marked reaction to some group of pollens other than the *Compositae* family (the group to which the ragweeds belong). In other words, it appears that half of the cases sensitive to ragweed, which have no symptoms until the season of ragweed bloom, showed a skin reaction that was markedly positive to pollen of other groups. While these patients have not been tested by the ophthalmic test, yet, in my judgment, in the majority of cases they would be found to have a sensitive mucous membrane to correspond with the skin sensitivity. It occurred to us a few years ago that the reason why poor results were obtained in the treatment of some of the seasonal hay fever cases

was due to the fact that the other pollens to which they are sensitive might be playing a secondary part. In other words, a patient might be rather markedly sensitive to one or all three of the pigweeds and yet have no symptoms until after the ragweed season. It is very difficult to desensitize a ragweed patient sufficiently to protect him against the pollen content of the air on the few days during the season when it is exceptionally high. On these days the mucous membrane of the nose will be badly irritated and from that time on the pigweed pollen would play a very definite part in keeping up symptoms. In other words, after the nose has been injured by the ragweed pollen it is an abnormal membrane, so to speak, and then even mechanical dusts and chemical odors and the cool air will irritate it sufficiently to produce at times marked symptoms. Believing our idea just mentioned to be a correct one, during the last two years we treated a number of seasonal hay fever cases which were sensitive to ragweeds, which were also sensitive to a group of pollens other than ragweeds, but whose symptoms did not appear until the ragweed season of bloom. These patients were treated both with the ragweed pollen extract and an extract from another group of pollens, to which they were found specifically sensitive.

CASE 1.—Dr. J. A. H., forty-eight years of age, came to the Clinic complaining of seasonal hay fever of six years' duration. He had been desensitized two seasons prior to coming to us by using a ragweed extract and through the guidance of a most excellent man doing allergic work.

On testing him he was found sensitive to the following:

Giant ragweed	++++
Short ragweed	++++
Western ragweed	++++
Bermuda	++++

He had never manifested symptoms until the fifteenth day of August. I suggested to him the possibility of Bermuda grass being a definite factor after the ragweed season appeared, and outlined his treatment accordingly; namely, using both the ragweed extract and Bermuda, but not mixed, of course, starting the treatment at such a time that both products would be built up to a maximum dose before the fifteenth of August, and excellent results were obtained. This has been repeated two years since with similar results.

It is very common with us to find cases of seasonal hay fever without symptoms until August 15, extremely sensitive to one or more of the amaranths or one or more of the grasses. In these cases we use routinely in treatment both ragweeds and the amaranths, or both ragweeds and grasses, or in some cases sensitive to ragweeds, the amaranth family and the grasses, we use all three groups. We feel that by so doing our results have been materially better.

RÔLE PLAYED BY ANIMAL DANDER IN UNCOMPLICATED SEASONAL HAY FEVER

A patient born with the ability to become sensitive to pollen and having seasonal hay fever theoretically should become sensitive to other atopic substances, especially if the familial tendency is great. Our findings lead us to believe that this is true since out of the 719 cases under consideration, 254, or 35.3 per cent, gave marked skin reactions to animal dander. A patient who suffers from seasonal hay fever frequently complains of the irritation of cold

air, burned bacon, smoke of any kind, etc. In other words, after the nasal mucous membrane has been made irritable by the pollen to which he is specifically sensitive, even nonspecific substances have the ability to produce an outpouring of mucus. If this be true, then it seems much more likely that patients with seasonal hay fever who are also sensitive to animal dander, would have their nasal mucous membrane irritated by the animal dander during the pollen season. It is my opinion that the animal dander to which seasonal hay fever patients are sensitive may cause no symptoms out of season, but after the nose has been irritated by the pollen, the animal dander then becomes a very definite factor.

CASE 2.—Mrs. B. W. M., aged forty-two years, complained of seasonal hay fever for a period of ten years.

On testing, a marked sensitivity to the following was found:

Giant ragweed	++++
Short ragweed	++++
Goose feathers	++++
Duck feathers	+++

Pollen therapy was instituted with only fair results. We then elicited a history of sneezing at night, and she was advised to eliminate feather pillows from her bedroom.

The next season pollen therapy was instituted, with the elimination of feather pillows from her bedroom, with excellent results.

We have had similar experiences in many other cases, which leads me to believe that feathers are a common secondary factor in seasonal hay fever, and that therefore feather extract should be used routinely in testing seasonal hay fever patients. If they are found sensitive to feathers, feathers should be removed from the bedroom at least during the pollen season.

ORRIS ROOT AS A SECONDARY FACTOR IN SEASONAL HAY FEVER

It is not uncommon to find seasonal hay fever patients who show a marked skin reaction to orris root but have only slight or no nasal symptoms outside the pollen season. Of the 719 cases, 75, or 10.4 per cent, were found to have a skin strongly positive to orris root by testing. It is only reasonable to believe that seasonal hay fever patients who show a positive reaction to orris root and pollen, but who have no symptoms relative to orris root out of season, should have orris root symptoms after the season appears and the nose has been made irritable by the pollen. Our own experience in many cases has proved the truthfulness of this assumption.

CASE 3.—Miss L. A., aged twenty-four, complained of seasonal hay fever of three years' duration, beginning September 1, with no symptoms at any other time of the year.

On testing she was found sensitive to the following proteins:

Giant ragweed	++
Short ragweed	+++
Western ragweed	++++
Orris root	+++

Desensitizing with the three ragweeds was done, with about 50 per cent relief. She had been advised to eliminate her orris root, which she had not done. After poor results were obtained during the season the orris root was finally eliminated, with almost immediate relief.

This patient is one of many whom we have seen, most of them females, who are sensitive to both pollen and orris root, on whom we obtained poor results with pollen therapy alone and good results when both orris root and the pollens were used in desensitizing, or when orris root was thoroughly eliminated during the season. If a patient has mild symptoms due to a sensitivity to orris root, it is the author's judgment that desensitizing with orris root should be done along with pollen therapy, and it should be instituted prior to the season, at such a time that the maximum dose of orris root can be reached before the patient's hay fever season.

SEASONAL HAY FEVER CASES ALSO SENSITIVE TO FOOD

Forty-four, or 6.1 per cent, of the 719 cases of pollen seasonal hay fever were very definitely sensitive to one or more food proteins. Of these cases a number were treated without eliminating the foods to which they were specifically sensitive, with unsatisfactory results, only to find the results much better on eliminating the foods, either during the same season or the following season.

CASE 4.—V. L. H., a girl, aged six, complained of seasonal hay fever of a very severe type, beginning August 15. No symptoms relative to the respiratory tract appeared prior to this time. She had eczema which had existed since infancy, however. Desensitizing had been done without relief.

Desensitizing, along with the removal of the foods from the diet, not only relieved her hay fever but eliminated her eczema.

Until the last two or three years it was generally believed that asthmatic or seasonal hay fever cases, sensitive to foods but without evidence of urticaria or eczema, should be desensitized with, or should avoid, the inhalants to which they were sensitive, but removal of the foods to which they were sensitive was not considered important. We have seen a number of cases, however, in both seasonal hay fever and asthma, who were desensitized to, or avoided the specific inhalants, with poor results, on whom we obtained excellent relief after eliminating from the diet the foods to which they were sensitive.

Although little has been written concerning the secondary factors in seasonal hay fever, I am sure that every allergist has been impressed with the importance of such factors in seasonal hay fever. The value placed upon the secondary factor, however, probably has been underestimated. It is my judgment that the part played by secondary factors is not an uncommon cause of poor results in the treatment of seasonal hay fever.

From our observation we must conclude that seasonal hay fever patients should be routinely tested with the common animal danders and other dusts, and the common foods, along with the pollens, and that if a marked skin reaction is found to the other inhalants or foods, they should at least be eliminated during the hay fever season.

We are also led to believe that many seasonal hay fever patients require desensitization against one or more groups of pollen other than the one whose season of bloom corresponds to the period over which the patient suffers.

MEDICAL ARTS BUILDING.

THE HINTON GLYCEROL CHOLESTEROL AGGLUTINATION REACTION*

Modifications in Technic (Second Communication)

By William A. Hinton and Genevieve O. Stuart,† Boston, Mass.

IN JUNE, 1927, a new serum test for syphilis was described and the results of its application on 138 syphilities and 368 nonsyphilities reported.[1]

Upon further use of the test, unforeseen difficulties arose. During the summer when the room temperature was as high as 32° C., it was troublesome to keep the bath at 27° C. for the necessary period of incubation. Even when this obstacle had been overcome by the use of a specially constructed water-bath, poor reactions would occur occasionally. Upon further investigation, it was found that chilling the reagents to a temperature lower than 27° C. before they were mixed gave more uniform results. Notwithstanding these changes, sometimes serum from a known syphilitic which gave positive Wassermann and Kahn reactions would give a negative Hinton test.

In the hope of overcoming this difficulty, use was made of the observation that the smaller amounts of serum from some syphilities gave a stronger reaction than the larger. Consequently, all serums were titrated routinely. The smallest quantity used was decreased from 0.1 to 0.0125 c.c. and the largest increased from 0.3 to 0.5 c.c. Serums from syphilities thus titrated, which previously had given negative Hinton though positive Kahn and Wassermann reactions, frequently gave strongly positive reactions with the smaller amounts of serum. An analysis of several hundred serums showed that there were three main types of reaction: (1) those serums with a strong reaction in all amounts; (2) those with the strongest reaction in the largest amounts, and (3) a few with the strongest reaction in the smallest amounts. This "zone" phenomenon, therefore, appeared to be a characteristic of the reaction.

Even after the changes described above, the clearing of the suspension in the positive cases occasionally was not pronounced, and therefore the agglutination of the dispersed particles of cholesterol was incomplete. Consequently, the period of incubation was prolonged to forty hours (at 27° C. as formerly) in order that the sensitiveness of the reaction might be increased and the difference between the positive and the negative reactions appear more definite. Much better results were obtained, as clinical investigation demonstrated. But such a long period of incubation was not so practical.

Accordingly, use was made of the observation that when the temperature was gradually raised to 37° C. or even to 42° C., after an initial period of incubation of five or six hours at 27° C., the reaction was much more differentiated. Therefore, incubation at 37° C. for a period of sixteen hours was tried and the

*The phenomenon is one of agglutination and not of precipitation, to which reference was made in the first communication.

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readings were compared with those where the temperature had been 27° C. and the period of incubation forty hours. The tests compared extremely well in the corresponding tubes where 0.1 c.c. of serum or less was used, but in those where 0.2 c.c. of serum or more was used, there was somewhat more clearing and therefore less differentiation between the positive serums and some of the negative serums, if clearing alone were the basis of differentiation. In the original description of the test, clearing alone was considered an indication of a positive reaction; but with the modified technic to be described, the criterion of positivity is based on the more certain factor, namely, *visible agglutination* of the dispersed particles of cholesterol. In strongly reacting serums, the particles of cholesterol will agglutinate into one or several large clumps suspended near the surface of an absolutely clear fluid, while the negative reactions are manifest by an even turbidity, or even by a distinct clearing of the fluid, but the absence of definite agglutinated masses of cholesterol.

We had often noted that clearing of the suspension, with resulting granularity, occurred frequently in the tubes containing the larger amounts of serum. This granularity, which might be mistaken for a weakly positive reaction, we were able to minimize by adjusting the proportion of muscle extract and cholesterol used in the preparation of the stock indicator. This adjustment is empiric and will be described later.

Furthermore, it was found that the optimum amounts of serum to be used with the modified technic were 0.1 c.c., 0.3 c.c., and 0.5 c.c., rather than 0.1 c.c., 0.2 c.c., and 0.3 c.c., as previously stated.

The technic which we use at present ensues:

The following reagents are required, and it is essential that clean, dry glassware be used in their preparation:

1. A 5 per cent solution of sodium chloride (C.P.) in distilled water.

2. A 50 per cent solution of glycerol, made by mixing equal parts by volume of distilled water and redistilled neutral glycerol.

3. A 0.7 per cent solution of cholesterol (Merck) in absolute alcohol. For rapid and complete solution of the cholesterol, place in an incubator at 37° C. or even 56° C.

4. An ether-insoluble, alcohol-soluble extract of beef muscle, referred to as the "activating extract," and prepared as follows: Round beefsteak, freed from fat and connective tissue, is finely ground and dried for four or five days at 56° C. in a large, flat dish. The dried muscle tissue is then finely pulverized with a meat grinder, using the machine's finest disc. Next, the powdered tissue is extracted four or more times with ether (U.S.P.) in a glass-stoppered bottle, in the proportion of 1 gm. of the powder to 4 c.c. of ether for each extraction. This process is completed when the last extract is colorless. Vigorous shaking by hand for ten minutes is required for each extraction. Finally, all the ether is poured off and the residue, which consists of the ether-insoluble constituents, is dried on filter paper. This is extracted in a glass-stoppered bottle with 95 per cent alcohol for three days at room temperature, in the proportion of one part by weight of the dried, ground residue to five volumes of 95 per cent alcohol.

5. Stock indicator, prepared by mixing 1 part of No. 4 (*the activating extract*) with 9 parts of No. 3 (the 0.7 per cent solution of cholesterol) and

allowing the mixture to stand in a glass-stoppered bottle for from four to eight weeks to ripen. Frequently these proportions must be changed by the addition of more of No. 3 (the cholesterol solution) to minimize granularity with nonsyphilitic serums in the test itself. Our method has been to prepare *glycerinated indicator* (No. 6 below), using *stock indicator* (as prepared above) for comparison in actual tests with glycerinated indicator containing a smaller concentration of No. 4. This has been done by taking a fraction of the stock indicator (1 part of No. 4 and 9 parts of No. 3) and adding to it 0.1 of a part more of No. 3. From this a glycerinated indicator is prepared and compared with the glycerinated indicator made from the stock indicator, in order to observe the relative amount of granularity which each shows with 10 or more known nonsyphilitic serums while the reaction obtained with an equal number of known syphilitic serums is moderately or strongly positive. This procedure is repeated, using 0.2 of a part of No. 3 instead of 0.1 part, and then again by using 0.3 of a part of No. 3, and so on until that proportion has been obtained which gives the minimum amount of granularity in the negative serums and the maximum number of positive reactions with syphilitic serums. The optimum proportions for most of our extracts have been 1 of the activating extract plus 10 of the 0.7 per cent cholesterol solution. The solution thus prepared is called the *adjusted stock indicator*. It appears to keep indefinitely.

This adjustment is required for accurate work.

6. *Glycerinated indicator*, freshly mixed for each day's test, is made as follows: One part of the *adjusted stock indicator*, composed of 0.7 per cent cholesterol and the ether-insoluble, alcohol-soluble fraction of beefsteak muscle, is pipetted into a glass-stoppered graduated cylinder, and 2 parts of the 5 per cent solution of sodium chloride added. The graduate is stoppered quickly and shaken vigorously for three minutes. Next, 12 parts of the 5 per cent solution of sodium chloride are added and the contents shaken thoroughly. Finally, 15 parts of the 50 per cent glycerol solution are added, and the whole is mixed well. It is important to follow these directions precisely.

In addition to these reagents and the glassware necessary in their preparation, the following are required:

1. Test tube racks which hold 30 or 60 tubes are desirable; 10 or some multiple thereof in a row will avoid confusion.

2. Small test tubes 10 mm. in diameter and 100 mm. long, commonly called serum tubes. It is important that the diameter of the tubes be fairly uniform, not exceeding 11 mm., because totally incorrect results may occur with the use of larger or smaller tubes.

3. An inactivating bath kept at a temperature of 55° C.

4. An incubator or Wassermann bath, preferably the latter, kept at a temperature of 37° C., or a degree lower, but the temperature should never be higher than 37° C. This should be determined by the use of an accurately standardized maximum and minimum thermometer.

The test is conducted in the following way:

1. The serums should be heated at 55° C. for one-half hour, preferably just before they are tested.

2. With a 1 c.c. pipette graduated in one-tenths, pipette into the first tube 0.1 c.c. of serum, into the second 0.3 c.c., and into the third 0.5 c.c.

3. With a 10 c.c. pipette, add 0.5 c.c. of the glycerinated indicator to each tube.

4. Shake the rack by first inclining it so that the tubes are almost horizontal and then thrusting it quickly forward and backward. The first motion is as if one wished to throw the tubes from the rack. By repeating 10 times, one obtains a quick but thorough mixture of the serum with the glycerinated indicator. The presence of distinct foam in each tube is the only safe criterion of adequate mixing. This is an important step in the procedure.

5. Place the rack containing the tubes in a Wassermann bath or incubator, maintained at 37° C. (it may be slightly lower but no higher) and let it remain for sixteen hours (conveniently from 5 P.M. to 9 A.M.). Care should be taken not to disturb the contents of the tubes during or after incubation.

6. The results should be read within an hour. Negative reactions manifest themselves by a turbidity uniformly distributed throughout the fluid or even a clearing of the fluid, but no sizable agglutinated masses. Clearing with no agglutination indicates that the temperature has been too high, or the incubation period too long, or both. Weak or moderate positive reactions show a water-clear fluid, throughout which may be suspended many small or a few large clumps of agglutinated particles of cholesterol (recorded "W" or "M"). Strong positives show from one to several large clumps near the surface of the fluid (recorded "+"). Neither opalescence occasionally encountered in serum, nor hemolysis, unless marked, has affected the accuracy of the test. Where two or more tubes show a weak reaction, the result is reported as "doubtful." With experience in technic, the percentage of doubtful reactions is very small. One must remember that a certain number of serums will give a stronger reaction in the 0.1 c.c. amount than in the 0.3 or 0.5 c.c. amounts. These are reported as positive.

The technic described above may be applied to unheated spinal fluids. The quantities of spinal fluid pipetted for each specimen are 2 c.c., 1½ c.c., 1 c.c., 0.5 c.c., and 0.1 c.c. To each amount 0.5 c.c. of the *glycerinated indicator* prepared with the 5 per cent salt solution is added. The results should be read after a period of sixteen hours at 37° C. and again after a period of twenty-four hours at room temperature, because some of the positives are manifest only after the longer period. Spinal fluids never give granular reactions unless they are positive; hence the slightest changes are interpreted as positive. We have examined 100 spinal fluids and have found that the results are practically parallel with those of the Wassermann. A detailed analysis of these results will be the subject of another communication.

SUMMARY

Modifications have been described in the technic of a seroagglutination reaction for syphilis which is applicable to both serums and spinal fluids.

REFERENCE

1. Hinton, William A.: A Glycerol-Cholesterol Precipitation Reaction in Syphilis, Boston M. & S. J. 196: 993-996, June 16, 1927.

THE QUESTION OF REFLEX VOMITING FROM THE HEART INDUCED BY DIGITALIS BODIES*

By Melvin Dresbach, M.D., and Kenneth C. Waddell, M.D., Albany, N. Y.

WHETHER digitalis induces emesis by central or peripheral action is doubtless a question which many believe was settled long ago. The matter is not so simple, however, and is yet a point of considerable importance. Lately it has been brought to the fore by Drs. Hatcher and Weiss,[1, 2] their thesis being that digitalis bodies induce emesis by stimulating afferent nerve endings in the heart. The argument is based on experiments extending over a number of years and is the outcome of much thought devoted by Prof. Hatcher and associates to the subject of vomiting in its various aspects. Nevertheless, in spite of all that has been written about it, the vomiting reflex is still obscure in certain pharmacologic details as well as in other aspects. In this connection it must be said that Dr. Hatcher and his colleagues have done an important service in publishing the results and conclusions from their extensive researches.

Briefly, the conclusion of Hatcher and Weiss that digitalis emesis is due to the drug's action on the heart rests upon three observations made in the Cornell Laboratory: (a), that removal of the entire gastrointestinal tract does not prevent the emesis; (b), that denervation of the heart by operative means usually does do so; (c), that nicotine also prevents the emesis, apparently by the paralysis of the cardiac afferent nerve endings. The idea has also had considerable support from the clinical angle in the hands of Eggleston and Wyckoff,[3] so that the hypothesis appears to have a well established basis.

When, a short time ago, we discovered the powerful emetic action of k-strophanthidin† and that this substance is somehow rendered inactive in the body with relative rapidity, we had a means of studying repeated emesis induced by a substance which is closely related to digitalis both pharmacologically and chemically and which has a certain advantage in the safety with which it can be used to induce emesis. As we were interested in the experiments of Hatcher and Weiss[4] which first led them to conclude that the heart is the locus of action in digitalis vomiting, we naturally began to study the effects of strophanthidin and also true digitalis principles in animals with denervated hearts. Our results with strophanthidin were given out in 1926[5a] and were the first experiments to run counter to those of Hatcher and Weiss. In the meantime we have reexamined our data dealing

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†This is the noncarbohydrate portion of Kombé strophanthin obtained by hydrolysis of that body.

with emesis induced by digitalis bodies and have concluded to publish them, even though certain of our experiments have not been finished, because we are satisfied that our findings are sufficient to make it doubtful that digitalis emesis is due to action of that drug on the heart alone. That is all we attempt to show at this time.

The experiments, which have been published in detail,[5b] were all done on cats. While we used a large number of these animals in our first work on this problem, only twenty-two remained alive long enough to be serviceable after denervation of the heart. Sixteen of these were injected with two or more of the following substances; k-strophanthidin, ouabain, digitoxin, true digitalin, amorphous strophanthin and fluid extracts and tinctures of digitalis. In a total of forty-seven injections, either intraperitoneal or intravenous, only five failed to induce emesis. These sixteen cats had all been allowed various periods, amounting to days or weeks in some cases, for recovery from the denervation operation.*

Briefly, this was carried out in two ways: (a) The stellate ganglia were removed by an extrapleural method and the two vagi cut in the neck, one vocal cord having been excised to prevent suffocation and the whole operation requiring about an hour and a half; (b) the denervation was accomplished in three stages, with an interval of two weeks or more between them: the left vocal cord was removed; the thorax was opened, the stellates and much of the sympathetic chain excised and the right vagus cut; the left vagus was cut in the neck and the inferior cervical ganglion removed. By this longer technic a pretty thorough denervation of the heart was accomplished, and always plenty of time for recovery from the anesthesia was allowed before injections were made.

Another group of six cats was prepared by denervating the heart at one operation, instead of in several stages, and after an hour or two allowed for recovery they were injected intravenously with ouabain in an amount known to be emetic in the normal cat. Two of the six cats vomited. Two of the four which failed to vomit died and the remaining two were given a second dose and both vomited. The results in this group of six cats were not very decisive, therefore, though they did suggest that when the period of recovery from the operation was short, emesis was interferred with by the depression incident to the operation and anesthesia.

In the light of all of these experiments we concluded that digitalis bodies (including pure principles) can induce vomiting in animals after denervation of the heart, if they are given sufficient time to recover from the immediate effects of the operation on the cardiac nerves and from the anesthesia.

Hatcher and Weiss in their papers[1, 2] allude to the possibility of regeneration of nerve fibers severed in the denervation of the heart in our experiments. In five of our animals that was impossible, for they were used within forty days after the operation. According to Cannon[6] that would be too

*The denervation of the heart is discussed in our strophanthidin paper and in our recent article on digitalis emesis referred to above. Certain improvements in the denervation technic are necessary and these will be described in a later paper.

short a time for certain regeneration. Two others were used within sixty days. In the remaining nine, which we had to label as having a doubtful denervation of the heart, we do not know whether the individual fibers joining the cardiac plexus were afferent or efferent. We concluded, therefore, that the probability of impulses reaching the central nervous system from the heart in these nine cats was very slight.

Our results with strophanthidin have been interpreted by Hatcher and Weiss differently than by us and are regarded as supporting rather than refuting their contention that the emesis induced is of cardiac origin. As we believe that our experiments with strophanthidin have more of a bearing upon the problem of digitalis emesis than these writers are inclined to admit, we shall present the argument briefly at this point. Essentially, the reasoning of Hatcher and Weiss[1] is as follows:

"Suitable doses of nicotine abolish the emetic action of ouabain and that of intravenous injections of strophanthidin, because nicotine paralyzes the afferent endings in the heart in which those drugs act to induce vomiting. It is possible that nicotine blocks these afferent impulses in the ganglions.

"Nicotine does not depress the vomiting center when used in this way nor does it block all paths from the periphery, and it does not have a perceptible effect on the different endings in the peritoneum; hence it does not interfere with the emetic action of an intraperitoneal injection of strophanthidin. It is clear why larger intraperitoneal doses are required after the denervation of the heart than in intact animals. After the absorption of the smaller doses from the peritoneum, the drug acts on the heart of the normal cat to induce the vomiting reflex; after the cardiac nerves have been cut, such small amounts in the circulation act on the heart, but the impulses to the center are blocked and nausea or vomiting does not occur; with the larger doses, however, the irritant local action on the peritoneum induces vomiting."

In reply to the statements just quoted, we admit that the idea of nicotine exercising a selective action as indicated is a stimulating one, which furnishes a new point of view, but it seems to us that the employment of that agent in the problem under discussion is not without considerable risk. Nicotine, as is well known, has a complex action, and Hatcher and Weiss[2] admit that there is a possibility of depression of the vomiting center. While their experiments seem to show that no serious depression of the sort occurred, great caution is necessary in drawing conclusions as to the seat of the nicotine paralysis in the complex vomiting mechanism.

In regard to the next point which Hatcher and Weiss raise, namely, that larger doses of strophanthidin are necessary to induce emesis after denervation of the heart than before that operation, we must reply that the data, taken from our paper, on which they base the conclusion, are by no means numerous enough to justify it. Neither are we ready to admit that strophanthidin induces emesis after intravenous injection because it acts upon peritoneal nerve endings. There is a possibility of such action when the emetic is injected directly into the peritoneal cavity, but when it reaches the peritoneum via the blood stream, after having been injected in doses of 0.10 to 0.25 of a milligram per kilogram of body weight, it must be enormously di-

luted in comparison with the concentration of the emetic given directly into the cavity when the doses mentioned are dissolved in one to five cubic centimeters of fluid.

Hatcher and Weiss further suggest that inasmuch as we used hydro-alcoholic solutions of strophanthidin the alcohol might be responsible for the emesis, as it is known to be irritating to the peritoneum. That is true, but we have not been restricted to alcohol as a solvent but have used acetone instead. Acetone, in concentrations which we proved would not induce vomiting when injected into the peritoneal cavity, will hold strophanthidin in solution. Such a solution will induce vomiting just as readily as the alcoholic one.

Another matter which Hatcher and Weiss stress as evidence that strophanthidin acts peripherally and not centrally to induce vomiting is its failure to act directly on the center when applied by us to the floor of the fourth ventricle. We did only a few experiments of that kind and the results, as far as they go, fall in line with those of Hatcher and Weiss who could not induce vomiting by any of the digitalis bodies they applied to the region of the vomiting center. There are various explanations which could be offered for those results, both in our experiments and in theirs, but discussion of them would lengthen this communication beyond its proper limits. We shall deal with that subject in a later paper on digitalisemesis.

For the same reason we cannot enter here into a detailed argument against the view that strophanthidin and true digitalis bodies induce emesis by action primarily upon the heart. We shall merely summarize the experimental results which we have obtained: (a) The substances just mentioned induce emesis after denervation of the heart, providing that the animal is in good postoperative condition; (b) section of the spinal cord above the origin of the splanchnic nerves and below that of the phrenics does not prevent this emesis, again providing that the animal is in good postoperative condition; (c) section of both vagi does not prevent the emesis; (d) strophanthidin induces emesis in animals after denervation of the heart, no matter how it is administered, i.e., by mouth, by vein, subcutaneously, intramuscularly, and intraperitoneally. In view of these facts, it is difficult for us to conclude that any abdominal or thoracic organ is the seat of the emetic impulses. If we could cut off successfully all impulses from the abdomen and thorax and keep an animal in good condition, we might get more light on the problem. We hope to approximate that situation in future experiments.

Here we must emphasize the importance of postoperative depression as a disturbing factor in the vomiting reflex. Though Hatcher and Eggleston[7] recognized that any emetic may fail to act, owing to this sort of interference in a given animal, depression as a factor was minimized in the later work because, as Hatcher and Weiss[1] state, the far more severe operation of evisceration does not interfere with digitalis emesis. However, the evisceration experiments referred to[7] were done on dogs, and in their denervation work[4] cats were used. It does not follow that the vomiting mechanism in the one class of animals would suffer from the shock effect of an operation to the same degree as it would in another or that two operations of a quite dis-

similar character would result in the same degree of depression of the vomiting reflex in the two groups, or in either one of them.

As far as we are concerned our experience with cats certainly satisfied us that the denervation of the heart, no matter how carried out, will interfere with emesis for a variable length of time, and that this result is not entirely due to the anesthesia is shown by the fact that surgical anesthesia alone, maintained with ether for a time equal to that required for a denervation operation, will not prevent digitalis emesis if a few hours are allowed for recovery. We are inclined to think that division of the vagi is a thing which plays a big rôle and that considerable time is necessary for certain adjustments to take place in organs innervated by the vagi before the vomiting mechanism regains a normal, or approximately normal, condition. It will then react to digitalis bodies with positive nausea or emesis in nearly every case.

In closing we shall state in a few words our view regarding digitalis and strophanthidin emesis. As already mentioned, strophanthidin closely resembles digitalis bodies pharmacologically. This is shown by the marked emetic action of strophanthidin and by its action on the circulatory system.[8]

Doubtless the explanation of the similarity of effects is the fact of the chemical relationship which Jacobs and Hoffmann[9] demonstrated between strophanthidin and digitalis bodies. Our belief is, therefore, that all of these substances have a common seat of action in the induction of emesis and that it remains to be shown whether that seat of action, if there be only one, is central or peripheral.

SUMMARY

1. The problem of the origin of digitalis emesis is briefly reviewed and it is concluded that the theory of cardiac origin of this emesis does not rest on a secure foundation.

2. The main evidence against the view of cardiac origin is that denervation of the heart does not prevent the emetic action of any of the ordinary digitalis bodies nor of strophanthidin.

3. Since this emesis is not prevented by denervation of the heart, nor by division of the spinal cord just below the phrenic nerves nor by division of both vagi, the peripheral origin of the emesis is questionable or obscure.

4. The conclusion that nicotine abolishes the emetic action of ouabain and strophanthidin, and presumably of digitalis principles, by paralyzing cardiac afferent nerve endings is regarded as open to question.

5. The importance of postoperative depression as a disturbing factor in the problem is emphasized.

6. The emetic action of strophanthidin is discussed with special reference to that of the digitalis bodies. It is argued that these bodies and strophanthidin have a common seat of emetic action.

7. In the light of present knowledge, based on animal experimentation, it is not yet possible to say whether the emesis is of central or peripheral origin.

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THE SEDIMENTATION OF RED BLOOD CELLS*

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SINCE Fahraeus in 1918[5] revived the old observation that the red blood cells in blood obtained from patients with certain pathologic conditions settled with much greater speed than those secured from normal persons, a great literature, most of which has appeared in European journals, has accumulated upon the subject. Considerable enthusiasm has been displayed by numerous writers, while others, particularly in this country, have exhibited greater conservatism as to the value of the test in diagnosis and prognosis. Although the test appears to have considerable value in the study of certain pathologic conditions, particularly pulmonary tuberculosis, the clinical application is not the only feature to receive consideration. Interest in the test has involved primarily the mechanism whereby the rate of sedimentation is under certain conditions so markedly increased, and it is in connection with this aspect of the reaction that the following data are presented.

Almost as many theories, explaining the acceleration of the sedimentation rate in certain diseases, have been proposed as there have been authors who have reported upon the reaction. Fahraeus thought that it was due to a change in the electric charge of the red blood cells causing a loss of their repelling force and bringing about the production and agglutinates which settled more rapidly. However, he was unable to establish that theory by experimental evidence and later suggested that viscosity may be a factor. Others such as Jevons,[9] Hoeber,[8] Risse,[29] and Pohle[24] advanced ideas similar to the theory of electric changes of Fahraeus. Northrup and Freund,[18] and

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Oliver and Bernard[19] have shown that the stability of the red cell suspension is largely dependent upon the difference in potential between the cells and the medium in which they are suspended. If the potential difference falls to a certain point the red cells agglutinate. However, Pender[20] points out that calcium chloride and magnesium chloride reduce the potential difference, but the red cells do not agglutinate. A number, among whom may be mentioned Katz,[10] Plaut,[23] Starlinger and Froseh,[24] Plass and Rourke,[22] Abderhalden,[1] Levinson,[12] Linzenmeier,[14] Buscher,[3] Westergren,[36] and Alexander[2] believe that the increased sedimentation rate is due to the increased content of fibrinogen in the blood. Newham,[17] however, found that the fibrinogen content was normal in 14 cases which showed accelerated sedimentation. Schmitz,[33] Sachs,[31] Salomon,[32] and Fischel[6] thought that an alteration in the globulin-albumin ratio with an increase in globulin caused the increase in the rate of sedimentation. Wichels[37] favored the destruction of red blood cells as being the primary factor. An increase in the blood cholesterol as a cause for the acceleration was suggested by Kurten,[11] but this idea was opposed by Newham[17] who showed that the blood cholesterol was normal in 15 cases which exhibited accelerated sedimentation rates. Leendertz[13] believed that the presence of antibodies was the contributory factor, while Lohr,[15] and Herzfeld and Schinz[7] suggested that an alteration in the viscosity may cause the phenomenon. Others believe that the explanation does not lie in one factor alone, but that several factors collectively contribute to it. Morriss[16] suggests that the surface tension, capillary attraction and a difference in electric charges cause the acceleration. Pribram and Klein[26] think that increased viscosity and blood cholesterol may explain the reaction, while Popper and Kreindler[25] suggest that an increase of both fibrinogen and globulin offers the explanation. Cooper[4] believes that an increase in fibrinogen, cholesterol and globulin is the answer, and Petschacher[21] offers an alteration in viscosity and a high globulin content in the blood as a possible explanation. Rubin[30] thinks that the entire structure of the blood is altered so as to effect the characteristic acceleration in the rate of sedimentation. Puxeddu[27] provides evidence showing that the phenomenon is resident solely in the blood plasma while Newham[17] affirms that it is primarily located in the red blood cells. Wells, DeWitt and Long[35] have pointed out that after severe hemorrhages, the blood shows an increase in surface tension, and in tuberculosis the return to normal will be slower in proportion to the progress of the disease. Other investigators have observed changes in the total protein content, in lecithin, lipase, and phosphorus, as well as alterations in the size, number and hemoglobin content of the red blood cells of blood that shows an accelerated rate of sedimentation.

Manifestly, in view of such discordant opinions, the basic cause for accelerated sedimentation is obscure.

In connection with work which has been reported on the mode of action of formaldehyde in complement fixation systems,[28] it was observed that bacterial suspensions to which formaldehyde had been added were more difficult to throw down in the centrifuge than were those which had not been so

treated. Investigation of this point revealed the fact that the addition of formaldehyde increased the surface tension and, apparently, in that way sedimentation of the bacteria was made more difficult. The du Nouy surface tensiometer was employed and, in terms of dynes, the bacterial suspension showed a surface tension of 60 while the suspension with formaldehyde added to a concentration of 1:50 gave a reading of 67 dynes. These facts suggested the application of the surface tension principle to a study of the sedimentation of red blood cells.

The addition of formaldehyde to whole blood, to blood serum, to blood plasma, and to red corpuscles suspensions elevated the surface tension by about 5 dynes in each case even though as low a concentration as 1:250 was employed. Sedimentation tests were performed after a method which has been described by Morriss.[16] In certain tubes varying amounts of formaldehyde were mixed with the blood which was being tested. Upon making readings at regular intervals, it was evident that formaldehyde altered the rate of sedimentation of the red cells. A fairly strong concentration, 1:250, slowed the rate of sedimentation markedly, and weaker concentrations of formaldehyde permitted increasing rates of sedimentation up to that which was obtained when no formaldehyde was included in the test.

In view of the possibility that changes other than an altered surface tension might result from the addition of formaldehyde, a surface tension reducent, sodium oleate, was utilized and a similar series of sedimentation tests were performed in which the reducent was mixed with the blood before being drawn up in the pipettes. In this case it was assumed that the only changes effected by the sodium oleate were physical in character. The sedimentation rate of red blood cells was very markedly reduced by the addition of minute amounts of this reducent, so markedly, in fact, that after two hours, when the control showed a sedimentation of 10 mm., the sodium oleate tubes showed none whatever. This very remarkable alteration in the rate of sedimentation was interpreted as being due to the reduced surface tension. By altering the surface tension with this reducent almost any degree of sedimentation from zero to that exhibited by the untreated blood may be obtained.

Working on the assumption that variations in the sedimentation rate of red blood cells are due to alterations in the surface tension of the plasma, bloods were collected from patients with pulmonary tuberculosis, and sedimentation tests were conducted with and without sodium oleate, as seen in Table I.

In every case the sedimentation was reduced by sodium oleate to 2 mm. or less in two hours, whereas without the reducent the sedimentation was as great as 57 mm. However, the protective influence against sedimentation, afforded by the sodium oleate, appeared to be lost, at least partially, after twelve hours, for a number of specimens of blood, when close to that time interval, suddenly began to sediment rapidly. For example, in the interval between two and twelve hours, in one instance the cells settled from 1 mm. to 60 mm.; in another, from 0 mm. to 45; and in still another, from 1 mm. to 48 mm. At the twenty-four-hour period this phenomenon was even more

marked, so that at that time the readings were nearly the same with and without sodium oleate. The explanation of this observation has not yet been obtained.

It is interesting to note in passing that the case of pulmonary tuberculosis, classified as minimal A and that classified as moderately advanced A, exhibited such slow rates of sedimentation. (In the Table they are Numbers 4 and 3.)

TABLE I

TYPICAL RESULTS OF SEDIMENTATION TESTS PERFORMED UPON THE BLOODS FROM TUBERCULOUS PATIENTS AND NORMAL CASES, COMPARED WITH RESULTS OBTAINED WHEN SODIUM OLEATE WAS ADDED IN THE CONCENTRATION OF 1:2000

SPECIMEN NUMBER		SEDIMENTATION IN MM. PER 100 MM. HOURS					CLINICAL DIAGNOSIS
		0.5	1.0	2.0	12.0	24.0	
1	Native blood	3	15	30	46	47	Pulmonary tuberculosis, far advanced A.
	+ Sod. oleate	0	0	0	1	4	
2	Native blood	8	24	38	47	49	Pulmonary tuberculosis, far advanced B.
	+ Sod. oleate	0	0	0	1	43	
3	Native blood	0	0	1	5	12	Pulmonary tuberculosis, moderately advanced A.
	+ Sod. oleate	0	0	0	1	3	
4	Native blood	1	1	2	12	22	Pulmonary tuberculosis, minimal A.
	+ Sod. oleate	0	0	0	2	3	
5	Native blood	9	24	39	55	57	Pulmonary tuberculosis, far advanced B.
	+ Sod. oleate	0	0	0	4	29	
6	Native blood	22	42	57	63	64	Pulmonary tuberculosis, far advanced C.
	+ Sod. oleate	0	0	1	60	60	
7	Native blood	14	32	45	57	58	Pulmonary tuberculosis, far advanced B.
	+ Sod. oleate	0	0	0	5	16	
8	Native blood	4	9	19	40	44	Pulmonary tuberculosis, far advanced A.
	+ Sod. oleate	0	0	1	3	11	
9	Native blood	8	22	41	54	56	Pulmonary tuberculosis, far advanced A.
	+ Sod. oleate	0	0	1	25	45	
10	Native blood	0	1	2	3	4	Normal.
	+ Sod. oleate	0	0	0	0	1	
11	Native blood	20	36	48	55	55	Pulmonary tuberculosis, far advanced B.
	+ Sod. oleate	0	0	0	45	48	
12	Native blood	5	11	26	53	55	Pulmonary tuberculosis, far advanced C.
	+ Sod. oleate	0	0	1	48	48	
13	Native blood	0	2	6	20	30	Normal
	+ Sod. oleate	0	0	0	0	1	

It is evident, therefore, that a minute amount of sodium oleate, through its action as a surface tension reducent, very markedly decreases the rate of sedimentation of red blood cells in normal as well as in pathologic cases. In a number of instances the plasma was replaced by serum and the same rate of sedimentation was obtained in each case, which suggests that the fibrinogen factor in accelerating sedimentation is negligible.

Studies have been made upon specimens of blood from patients with pulmonary tuberculosis in varying stages, with the du Nouy surface tensiometer, but without detecting any very marked alteration in the surface tension. It is entirely possible that the changes are those of an interfacial character and not readily demonstrable by the apparatus. However, upon determining the surface tension of a specimen of plasma, it was found, for example, to be 61.7; with added sodium oleate, 52.4; and with added formaldehyde, 63.6. Nevertheless, there does not appear to be any other interpretation of the effect of sodium oleate upon the rate of sedimentation than that of an alteration of surface tension and it appears very probable that, since this reducent slows the sedimentation of blood from tuberculous patients through its effect in lowering the surface tension, the explanation of the accelerated sedimentation lies in an increased surface or interfacial tension. The cause of the alterations of the surface tension in the blood of tuberculous patients is problematical.

CONCLUSIONS

1. The surface tension of blood is increased by the addition of formaldehyde and is markedly decreased by the addition of sodium oleate.

2. The rate of sedimentation of red blood cells is greatly diminished by sodium oleate in normal bloods as well as in bloods obtained from patients with pulmonary tuberculosis, suggesting that the acceleration in rate of sedimentation in tuberculosis and other infectious diseases is explainable upon the basis of surface tension.

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THE TOXIC CONSTITUENT OF THE BILE*

By William C. Emerson, M.D., Detroit, Michigan

THE fact that bile is toxic has long been observed. Bunting and Brown[1] in experimental work on rabbits showed that by either injecting bile intraperitoneally or by dividing the gall bladder and allowing the bile to escape into the peritoneal cavity that death occurred within twenty-four hours and was attributed to direct action on the myocardium.

There has long been a controversy as to just which constituent of the bile is toxic. Bouchard,[2] Plaesterer[3] and King and Stewart[4] all claim that the toxic constituent of the bile is the pigment. Rohrig,[5] Feltz and Ritter,[6] Leyden,[7] Rywosch,[8] Stadelmann,[9] and Bickel[10] all maintain that the toxic constituent of the bile is the bile salts, sodium glycocholate and sodium taurocholate. Cholesterin and mucin surely are nontoxic.

Biedl and Krause[11] have shown that the bile salts are strongly hemolytic for blood and that in sufficient concentration they cause a coagulation of myosin to such an extent that the muscle loses its contractility. It is undoubtedly this action which causes death due to myocardial failure when whole bile or bile salts are absorbed by the blood over a long period of time. They also showed that when bile salts were injected intravenously, a slowing of the pulse with a marked fall in the arterial blood pressure occurred.

King and Stewart[4] prepared their pigment from pig's bile. We repeated their work and found that their pigment when dried to constant weight equaled 8.5 per cent. The pigment in dog's bile equals 0.05 per cent. We would not expect pig's bile to contain one hundred sixty times as much pigment as dog's bile. It would be reasonable to assume that their pigment contained other substances. Their dried pigment was analyzed and was found to contain bile salts and large amounts of fat.

This dried pig's pigment, the calcium having been removed by washing with hydrochloric acid, was extracted with hot chloroform, the chloroform evaporated to dryness and the residue dissolved in alcohol. This alcoholic solution of pig's pigment was injected into the femoral vein of a dog. There

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was no evidence of any toxic effect. Twenty-one c.c. of the concentrated solution, a 9 per cent solution of pigment dissolved in 95 per cent alcohol, was injected, and the blood pressure at the completion of the experiment was higher than at the beginning.

Meltzer and Salant[12] have shown that when a few cubic centimeters of ox bile was given rapidly to a rabbit intravenously the animal died almost immediately in convulsions, while, when given slowly, as much as 50 c.c. of a 50 per cent solution was tolerated. In all our work the material injected was given slowly 2.5 to 3 c.c. a minute.

METHOD

Ox bile was obtained from twenty gall bladders of freshly killed animals. The bile was diluted with an equal volume of normal saline and injected within three hours after collection. Dogs were used in all the experiments.

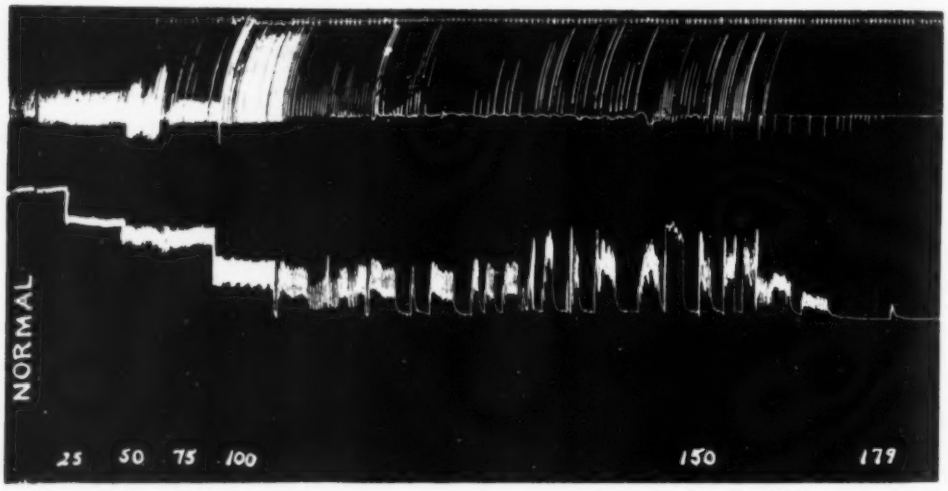

Fig. 1.—Effect on the blood pressure and respiration when bile is injected into the femoral vein.

The animals were anesthetized by the open drop method, later a cannula was placed in the trachea and connected with an ether bottle. A cannula was placed in the carotid artery and connected with a mercury manometer for blood pressure tracing. A tube was placed in the etherization system and connected with a tambour and writing lever for a tracing of the respiratory movements. The femoral vein was exposed and bile diluted with an equal volume of normal saline was injected from a burette. Blood pressure and respiration tracings were made after the injection of every 25 c.c. of the diluted bile.

A 3 per cent solution of sodium glycocholate was injected into the femoral veins of ten normal dogs. Blood pressure and respiration tracings were made as in the control experiments.

A 3 per cent solution of sodium taurocholate was injected into the femoral veins of ten normal dogs. Blood pressure and respiration tracings were made as in the control experiments.

The sodium glycocholate and the sodium taurocholate were prepared as follows: The commercial product of both was obtained. This material was recrystallized three times, the final product being a pure white powder. The melting point of the sodium glycocholate was 150° C. and the melting point of the sodium taurocholate was 95° C. This purified material was dissolved in distilled water and injected as described above.

On ten consecutive days ox bile from twenty freshly killed animals was obtained. The bile was diluted with an equal volume of normal saline and the mixture divided into two equal parts. One part, whole bile diluted with an equal volume of normal saline, was injected into a dog. The other half, also diluted with an equal volume of normal saline, was treated three times with blood charcoal and the final product, a clear light yellow liquid, was injected into a dog. This clear light yellow liquid when compared in the colorimeter against bile diluted with an equal volume of normal saline was found to contain only 5 per cent of pigment, the remaining 95 per cent having been removed by the blood charcoal. This experiment was repeated on ten consecutive days, ten samples of bile being used and twenty dogs, two each day, injected, one with whole bile and one with bile after 95 per cent of the pigment had been removed.

Pigment obtained from human gallstones was dissolved in alcohol and injected into a dog.

RESULTS

Ten specimens of bile were injected into the femoral veins of ten normal dogs. The lethal dose is summarized in Table I. The lethal dose for whole bile when injected into the femoral vein gave an average of 9.6 c.c. per pound. Fig. 1 shows the characteristic gradual fall in blood pressure which occurs when bile is injected into the femoral vein. The fall in blood pressure is later accompanied by a striking arrhythmia and at times there is an apparent cessation of cardiac activity.

TABLE I

DOG NUMBER	WEIGHT OF DOG POUNDS	LETHAL DOSE WHOLE BILE PER POUND C.C.
1	16.5	12.0
2	14.25	8.55
3	18.75	10.5
4	19.75	9.7
5	18.	10.2
6	12.5	8.4
7	15.75	8.77
8	10.25	10.5
9	19.	9.9
10	15.	8.0
Average		9.6

Three per cent sodium glycocholate was injected into the femoral veins of ten normal dogs. The lethal dose is summarized in Table II. The lethal dose of 3 per cent sodium glycocholate when injected into the femoral vein gave an average of 8.5 c.c. per pound. Fig. 2 shows the same gradual fall

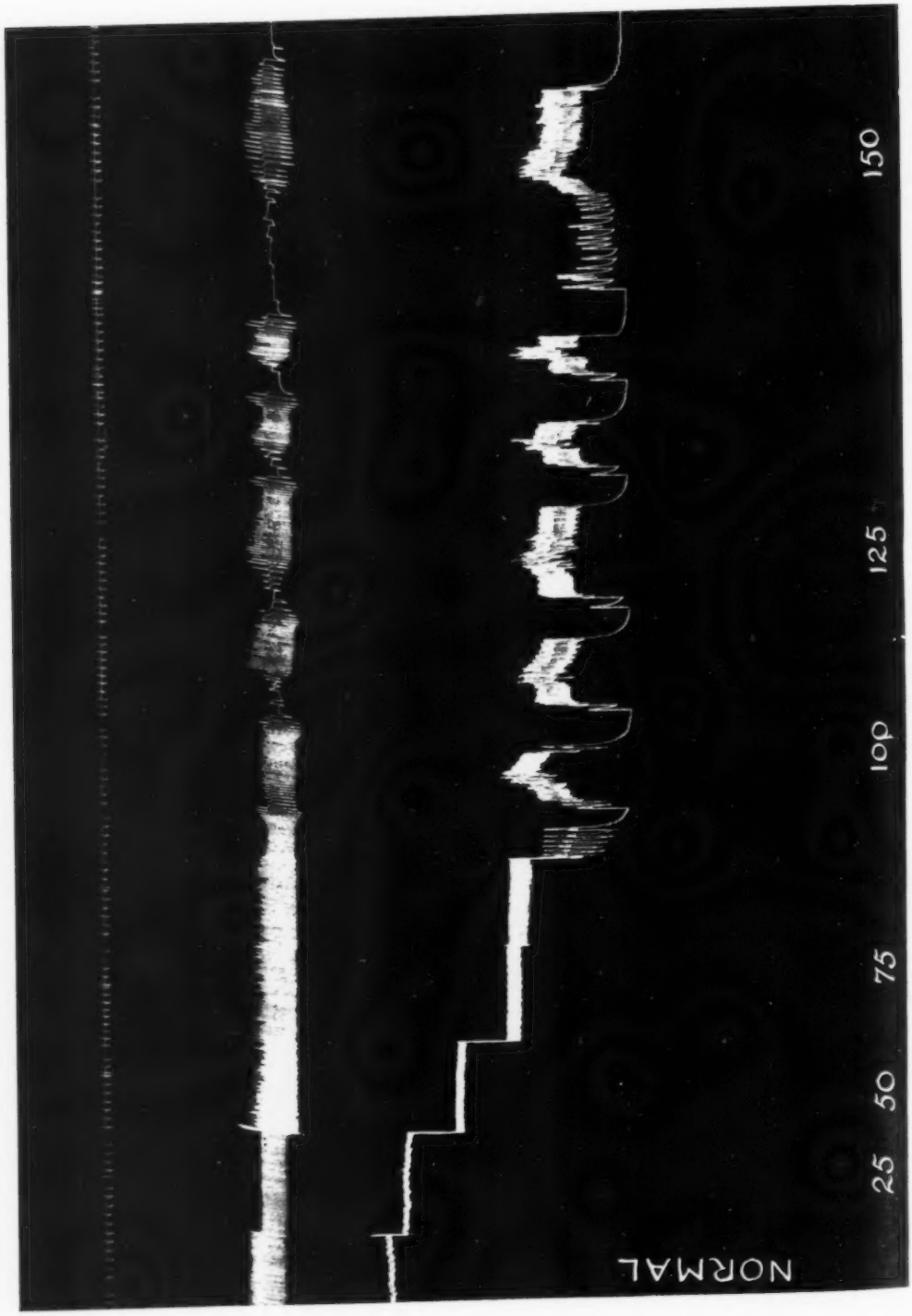

Fig. 2.—Effect on the blood pressure and respiration when 3 per cent sodium glycocholate is injected into the femoral vein.

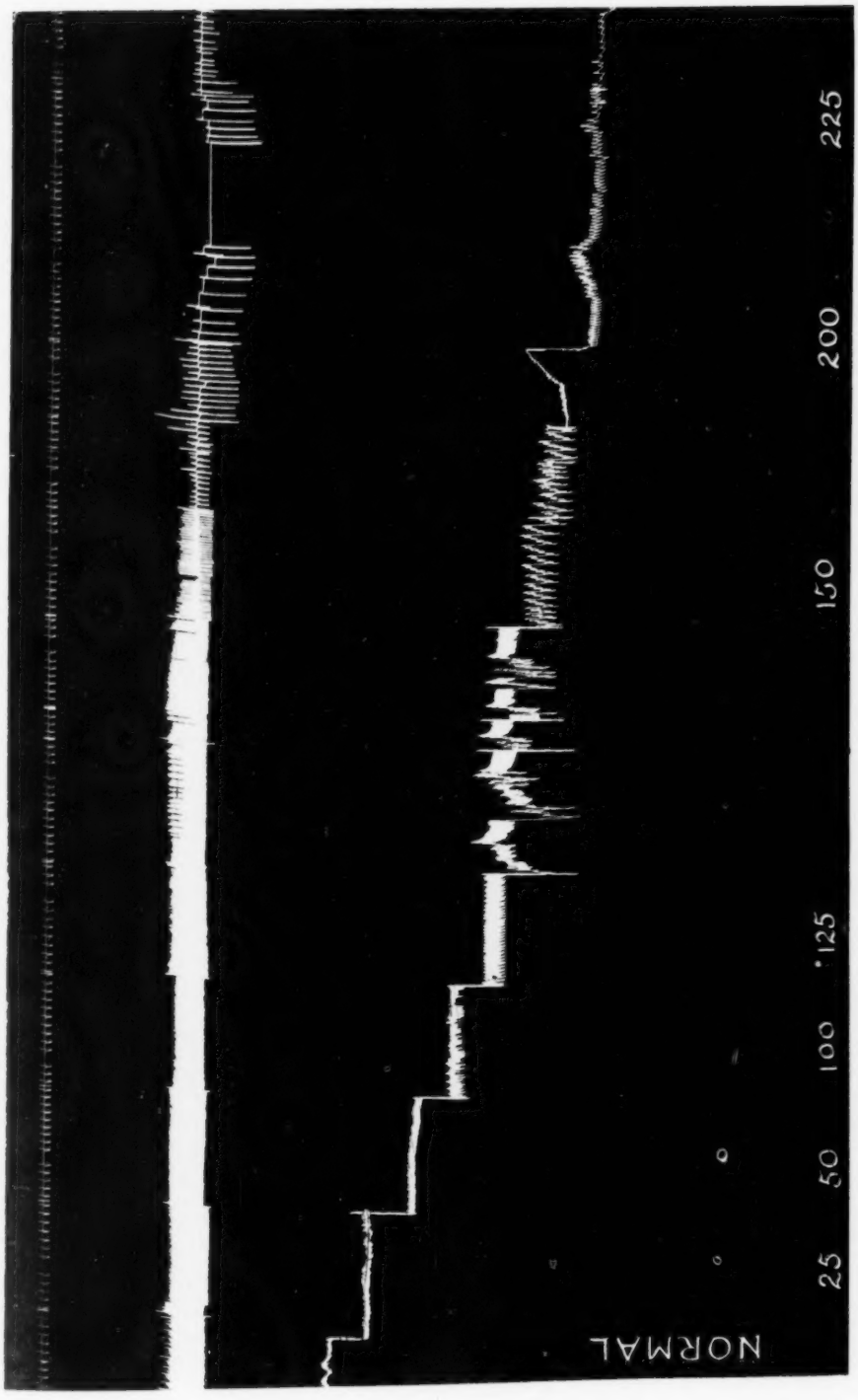

Fig. 3.—Effect on the blood pressure and respiration when 3 per cent sodium taurocholate is injected into the femoral vein.

Fig. 2.—Effect on the blood pressure and respiration when 3 per cent sodium glycocholate is injected into the femoral vein.

in blood pressure later accompanied by a striking arrhythmia as occurred in the control tracing when whole bile was injected.

Three per cent sodium taurocholate was injected into the femoral veins of ten normal dogs. The lethal dose is summarized in Table II. The lethal dose of 3 per cent sodium taurocholate when injected into the femoral vein gave an average of 10.1 c.c. per pound. Fig. 3 shows identically the same fall in blood pressure accompanied by a marked arrhythmia as occurred in the control tracing when whole bile was injected.

TABLE II

DOG NUMBER	WEIGHT OF DOG POUNDS	LETHAL DOSE 3% SODIUM GLYCOCHOLATE PER POUND C.C.	DOG NUMBER	WEIGHT OF DOG POUNDS	LETHAL DOSE 3% SODIUM TAUROCHOLATE PER POUND C.C.
11	18.5	8.0	21	19.0	12.0
12	18.0	7.5	22	7.5	10.9
13	11.5	6.0	23	21.25	6.6
14	14.0	7.8	24	17.5	6.2
15	14.25	8.07	25	14.5	10.4
16	17.5	9.9	26	13.0	10.0
17	10.5	9.9	27	9.25	10.8
18	19.0	9.5	28	12.5	11.2
19	21.0	9.19	29	13.75	11.4
20	14.0	10.2	30	9.5	11.5
Average		8.5			10.1

TABLE III

BILE SPECIMEN	WEIGHT OF CONTROL DOG POUNDS	LETHAL DOSE WHOLE BILE PER POUND C.C.	WEIGHT OF SECOND DOG POUNDS	LETHAL DOSE WHOLE BILE MINUS 95 PER CENT PIGMENT PER POUND C.C.
1	10.5	6.2	18.0	4.6
2	10.0	7.3	20.0	10.9
3	33.0	7.8	38.0	8.2
4	7.5	6.6	9.0	10.5
5	12.5	6.7	12.0	7.3
6	26.5	9.6	11.5	9.7
7	16.5	5.7	23.0	9.4
8	14.5	5.5	14.0	10.5
9	18.5	5.7	21.0	5.4
10	17.0	6.8	23.0	7.0
Average		6.8		7.3

Ten samples of ox bile were diluted with an equal volume of normal saline. Half of each sample was treated with blood charcoal. The treated bile after 95 per cent of the pigment had been removed and the untreated bile were injected into the femoral veins of twenty normal dogs, two dogs being used for each sample of bile.

The lethal doses are summarized in Table III. The average lethal dose for the untreated bile is 6.8 c.c. per pound. The average lethal dose for the bile after 95 per cent of the pigment had been removed was 7.3 c.c. per pound.

Fig. 4 shows the same fall in blood pressure accompanied later by the striking arrhythmia as occurred when whole bile was injected into the femoral vein.

Pigment obtained from human gallstones dissolved in alcohol was in-

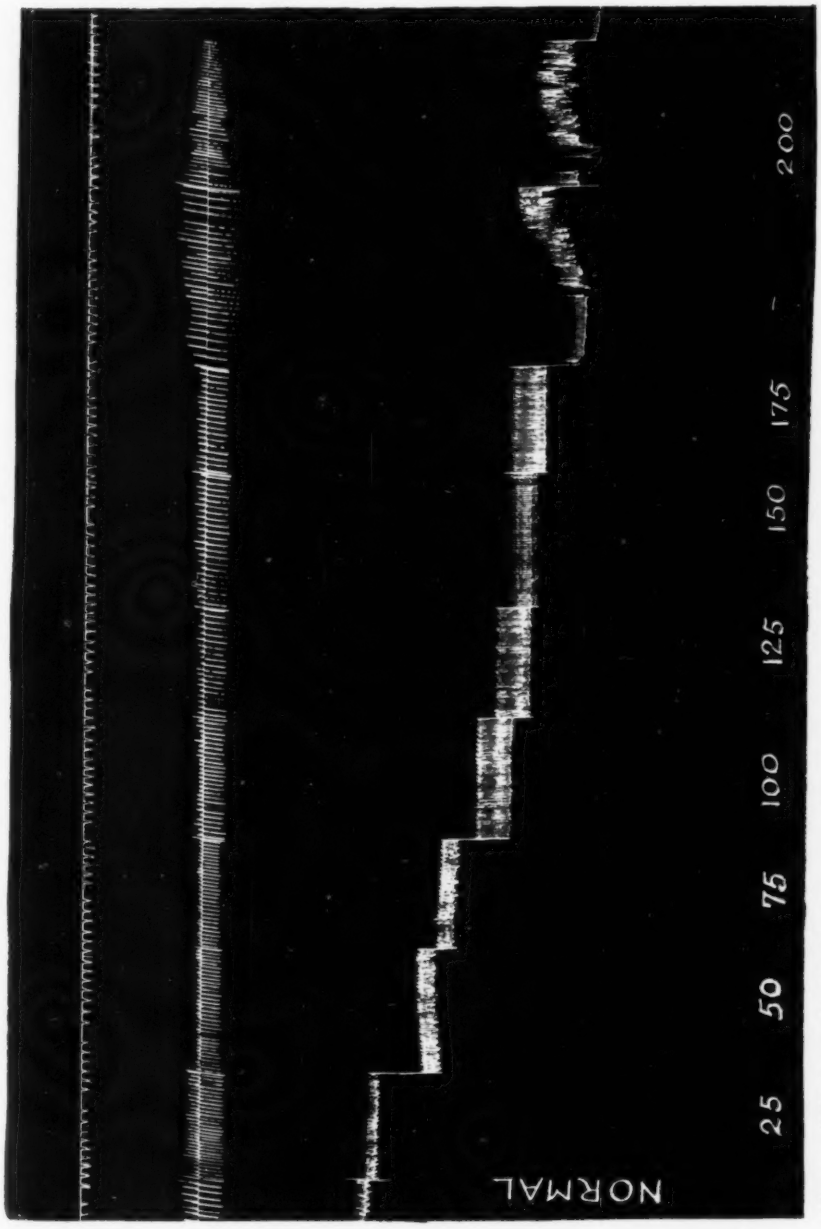

Fig. 4.—Effect on the blood pressure and respiration when bile after 95 per cent of the pigment had been removed was injected into the femoral vein.

jected into the femoral vein of a dog. There was no effect on the blood pressure or respiratory rate. Twenty-five c.c. per pound was injected, and the blood pressure was higher at the completion of the experiment than at the beginning.

DISCUSSION

The fact that the average lethal dose of sodium glycocholate injected at a uniform rate of speed is 1.1 c.c. per pound less than the average lethal dose of bile and that the average lethal dose of sodium taurocholate injected at a uniform rate of speed is 0.5 c.c. per pound greater than the average lethal dose of bile would suggest that the toxic constituent of the bile is the bile salts, sodium glycocholate and sodium taurocholate.

The blood pressure and respiration tracings of the dogs injected with sodium glycocholate and sodium taurocholate being identical with the tracing of the dog injected with whole bile would further indicate that the bile salts are the toxic constituents of the bile.

Sodium glycocholate is more toxic than sodium taurocholate due to the fact that the average lethal dose of sodium glycocholate is 8.5 c.c. per pound whereas it required an average of 10.1 c.c. per pound of sodium taurocholate to kill the animals.

The average lethal dose of bile after 95 per cent of the pigment had been removed being 7.3 c.c. per pound as compared to an average of 6.8 c.c. per pound when whole bile was injected would clearly indicate that the bile pigment is not the toxic constituent of the bile.

That the bile pigment is not the toxic constituent of the bile is further indicated by the fact that the tracing of the blood pressure and respirations in a dog injected with bile after 95 per cent of the pigment had been removed is identical with the tracing when untreated or whole bile was injected, and also by the fact that when pigment was injected there was no fall in blood pressure nor was there any arrhythmia produced.

CONCLUSIONS

1. The toxic constituent of the bile is the bile salts, sodium glycocholate and sodium taurocholate.

2. Sodium glycocholate is more toxic than sodium taurocholate.

3. Bile pigment is not toxic and has no effect on the blood pressure or respiratory rate when injected into the blood stream.

This work was suggested and done under the direction of Dr. Edward C. Davidson.

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THE EFFECT OF A SUDDEN INCREASE IN THE INTRACARDIAC PRESSURE UPON THE FORM OF THE T-WAVE OF THE ELECTROCARDIOGRAM

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THE effect upon the form of the electrocardiogram of a sudden rise in the intracardiac pressure within the left ventricle was investigated by clamping the thoracic or ascending portions of the aorta, and in the case of the right chamber by clamping the pulmonary artery in 19 experiments on dogs.† The effect of partially collapsing both the lungs, or twisting the hilus of one lung was also added. In order to prevent the onset of ventricular fibrillation a small dose of barium chloride (1 mg. per kilogram) or ouabain (⅓ lethal dose) was administered.

In five animals, clamping the ascending aorta was followed by a great increase in the rate and diminution of the voltage of the curve. In these the changes in the T-wave were not considered inasmuch as they were not primary. Of nine remaining animals in which the thoracic aorta was clamped, a positive influence upon the T-wave appeared in five. In the other four the procedure had no distinct effect upon the T-wave. This positive influence upon the T-wave was more pronounced where a strongly negative T-wave was present before. In one instance (Fig. 1) the effect was sufficiently intense to make the T-wave upright. This change in the direction of the T-wave was not permanent, and the electrocardiogram slowly resumed its original form. (Fig. 1.) The removal of the clamp was associated with the opposite effect, a negative influence upon the T-wave (Fig. 1, *c*).

In 5 animals observations upon the effect of alterations in the pressure in the lesser circulation were made. Twisting the lung hilus or incompletely clamping the pulmonary artery caused a negative effect upon the T-wave. A partial collapse of the lung, although giving the same result, was less certain in its effect. The effect, like that following the clamping of the aorta, was also of short duration, and the T-wave slowly returned to its original form (Fig. 3). When the pulmonary artery was completely occluded by the clamp, the rapid death of the animal was associated with greatly exaggerated negativity of the T-wave (Fig. 2). The same effect occurs in association with the

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†The animals were narcotised with chloretone, the vagi cut, artificial respiration instituted and the heart exposed by the removal of the sternum. Axial electrocardiograms were taken.

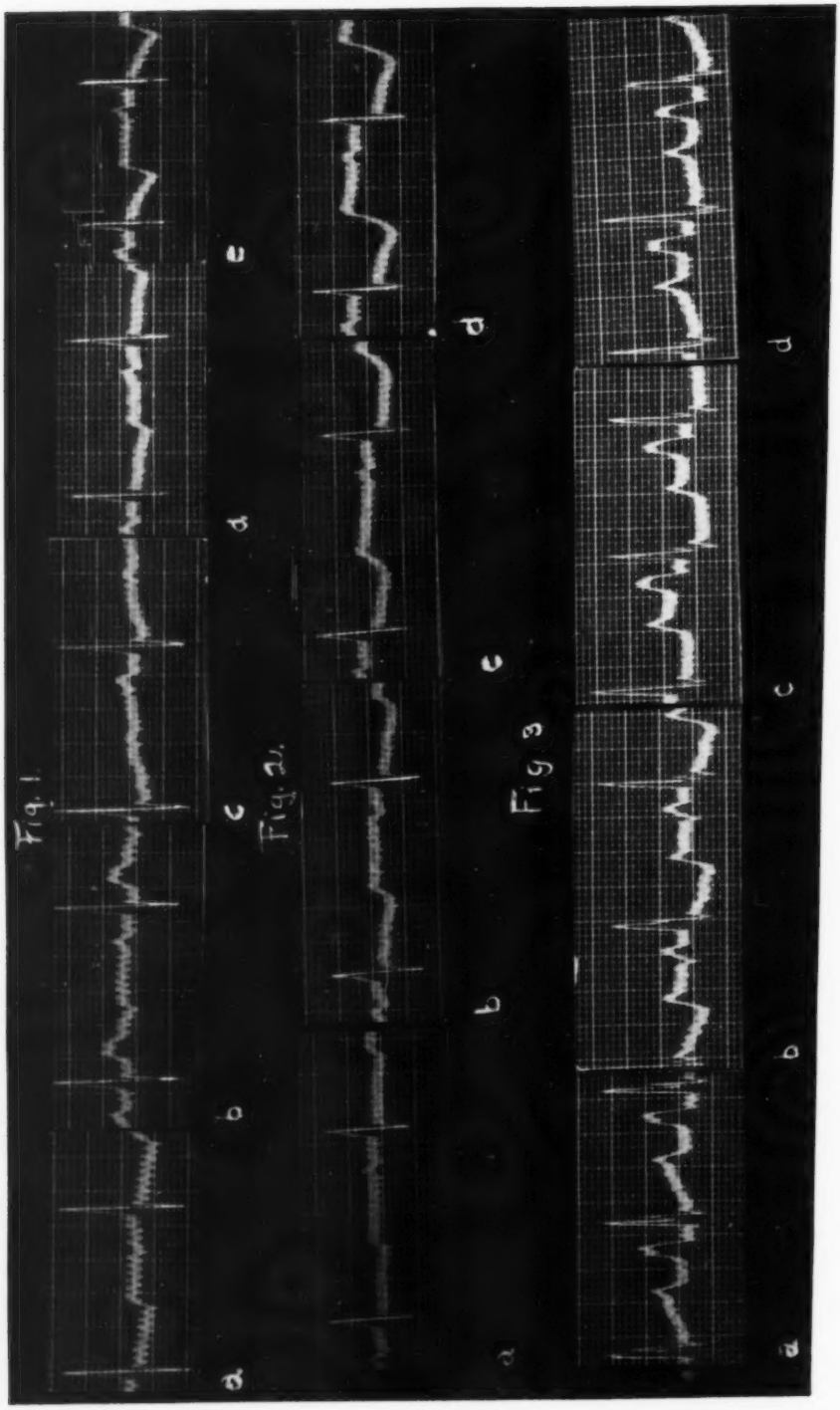
Fig. 1
Fig. 2.
Fig. 3

closure of the right coronary artery[1] and also, circulatory failure which is induced by any procedure causing great cardiac depression.

The changes in the T-wave were not produced by the very slight changes in the position of the heart with reference to the line of the leading which are unavoidably associated with the procedures used. The degree to which they shifted the position of the heart was without a significant effect upon the T-wave. If anatomic changes are the cause of the alteration in the T-wave, they are more likely to be the effects of the slight shift in the position of the septum without alteration of the gross position of the heart which results from the changes in the intracardiac pressure.

The human electrocardiogram taken in the presence of pneumothorax often presents changes in the T-wave and RT interval of the same nature.[2] These changes are much more apt to appear with acute spontaneous pneumothorax than with the artificial type and tend to lessen as the duration of the pneumothorax increases. The experiments bearing upon the pulmonary circulation suggest that the sudden alteration of the resistance in the pulmonary circulation caused by the collapse of the lung is the cause of the T-wave changes which occur in that condition.

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Fig. 1.—Axial (RA—LL) electrocardiograms. Time in fiftieth seconds. *a*. The normal electrocardiogram. *b*. After application of the clamp to the descending aorta just below the diaphragm. *c*. Five minutes later. *d*. Ten minutes later. The T-wave has resumed its original form. *e*. Just after removal of the clamp.

Fig. 2.—Axial electrocardiograms. Time in fiftieth seconds. *a*. The normal electrocardiogram. *b*, *c*, *d*. Electrocardiograms at short intervals after clamping the pulmonary artery. Death followed soon after.

Fig. 3.—Axial electrocardiograms. Time in fiftieth seconds. *a*. The normal electrocardiogram. *b*. After twisting and clamping the hilus of the right lung. *c*. Five minutes later. *d*. Five minutes later. The T-wave is again upright.

THE ACTION OF PHOSPHATES UPON THE ENDOCRINE AND SYMPATHETIC SYSTEMS*

By L. Loumos, M.D., Chicago

THE use of iodine in the prophylaxis and treatment of goiter and allied disturbances, also in the treatment of such diseases as, pulmonary tuberculosis, scrofula, lymphadenitis, bronchitis, asthma, etc., is well known. A great objection to the use of iodine in large doses, or in moderate doses for an extended period of time is the frequent appearance of iodism.

I have administered, in a large number of cases, iodine together with phosphates, and seldom, if ever, have I noted any symptoms of iodism so long as the phosphates were taken; therefore, the present investigation was undertaken in order to determine more definitely whether the use of phosphates increases the tolerance of iodine and prevents the manifestation of the symptoms associated with the administration of large amounts of iodine.

The fact that phosphates increase the tolerance to iodine and diminish considerably the frequency of the appearance of iodism has been noted by Huchard.[1] Iodine and phosphates appear antagonistic in their action, iodine being a stimulant, and phosphates depressant to the "basal metabolism activity." It was then thought advisable to investigate the action upon experimental animals.

To two dogs 0.2 gm. of iodine (in tincture form) was given daily for twenty days; in addition, to one of the dogs 1.0 gm. of equal parts of sodium phosphate (Na_2HPO_4) and calcium phosphate ($Ca_3(PO_4)_2$) were given daily. Both were kept on the same diet. The results are summarized in Table I.

Table I

INITIAL WEIGHT	TOTAL IODINE IN 20 DAYS	TOTAL PHOSPHATES IN 20 DAYS	WEIGHT AFTER 20 DAYS	REMARKS
21 lb.	4.0 gm.	0.0 gm.	17.5 lb.	First signs of hyperthyroidism on fourteenth day, then increased nervousness, ravenous appetite, etc.
20 lb.	4.0 gm.	20.0 gm.	21.0 lb.	No evidence of hyperthyroidism

Table II

INITIAL WEIGHT	TOTAL IODINE IN 40 DAYS	TOTAL PHOSPHATES IN 40 DAYS	WEIGHT AFTER 40 DAYS	REMARKS
18 lb.	4.0 gm.	0.0 gm.	16.1 lb.	First signs of hyperthyroidism on thirty-third day. Symptoms of moderate intensity
16 lb.	4.0 gm.	20.0 gm.	18.0 lb.	No evidence of hyperthyroidism

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The next step was to decrease the dose to 0.1 gm. of iodine and 0.5 gm. phosphates per day, and extend the time to forty days, repeating the above experiment on another set of dogs. The results are given in Table II.

As was pointed out above, the fact that the dogs to which phosphates were administered failed to show hyperthyroidism, is thought to be due to the depressing action of the latter on the basal metabolism activity. In this connection the elimination of iodine was investigated, and it was found that this is also retarded by the phosphates.

According to Kendall,[2] the normal blood iodine content is about 0.13 mg. This amount, however, varies, depending upon different causes such as diet or the condition of the thyroid gland. For example, in the cretin the circulating amount of iodine is decreased. Other authors note a difference in the amount during the late summer and autumn, and during the winter months.

Gley and Cheymol,[3] conducting their investigations on goats, found 0.12 mg. to 0.19 mg. while in our experiments on dogs (using Kendall's method), we found 0.11 mg. to 0.17 mg.

A number of determinations of the iodine content of the blood, with dogs taking iodine alone, and also with dogs taking phosphate in addition to iodine were made, and the results are summarized in Table III.

TABLE III

SPECIMEN	CONTENT OF IODINE MG. PER 100 C.C. OF BLOOD (KENDALL'S METHOD)
1. Dog: Given a total of 4.0 gm. iodine over a period of twenty days. Hyperthyroidism: 50 c.c. of blood was drawn from jugular vein twenty-four hours after last dose	0.194
2. Dog: Given total of 4.0 gm. iodine and 20.0 gm. phosphates over a period of twenty days. No evidence of hyperthyroidism: 50 c.c. of blood was drawn twenty-four hours after last dose	0.348
3. Dog: Given 0.1 gm. iodine and 1.0 gm. phosphates daily for twenty-five days. (Total 2.5 gm. iodine and 25 gm. phosphates.) No evidence of hyperthyroidism	
Analysis of blood:	
Before administration of iodine	0.170
Twenty-four hours after last dose	0.950
Fifteen days after last dose	0.240
Twenty-five days after last dose	0.160

The determination of iodine in the blood was then made during the administration of iodine as well as after. The same procedure was used as before; the dogs were on the same diet, and special care was taken in the amount of salts added to the food and the selection of the food. The results are summarized in Table IV.

In the interpretation of the above results it must be taken into consideration that the elimination of iodine is very rapid; from 65 to 80 per cent appears in the urine within twenty-four hours after its administration.

TABLE IV

SPECIMEN	CONTENT OF IODINE MG. PER 100 C.C. OF BLOOD (KENDALL'S METHOD)
1. Dog: 0.1 gm. iodine daily for twenty days	
Analyses:	
Before administration	0.146
Tenth day	0.152
Twentieth day	0.158
Ten days after discontinuance of administration	0.142
2. Dog: Given 0.1 gm. iodine and 0.5 gm. phosphates daily for twenty days	
Analyses:	
Before administration	0.128
Tenth day	0.181
Twentieth day	0.276
Ten days after discontinuance of administration	0.167

From Tables III and IV it appears that in the animals taking phosphates and iodine, there is a retardation of the elimination of iodine, and after the administration is discontinued it takes ten to twenty days for the return to the normal iodine content.

CONCLUSION

The retardation of the elimination of iodine, and the failure to cause experimental hyperthyroidism in animals taking iodine in conjunction with phosphates can be explained by the assumption that phosphates exert a depressing action upon the "endocrine and sympathetic systems" which control the function of basal metabolism.

The practical benefit derived from this combination of iodine and the phosphates is apparent, the danger of iodine is minimized, and at the same time the organism uses more iodine due to the slow elimination.

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LABORATORY METHODS

METHODS OF TESTING ANTISEPTICS*

By George F. Reddish, Ph.D., Baltimore, Md.

FOR a great many years the bactericidal efficiency of antiseptics have been determined by what is known as the "phenol coefficient test." In this country two tests have been used for this purpose, namely, the Rideal-Walker[1] and the Hygienic Laboratory[2] methods. The first named test was published in England in 1903 as a method for determining the germicidal value of disinfectants as compared to phenol. Since in England antiseptics are understood to be substances which kill microorganisms,[3] they also were tested by the Rideal-Walker method. It was consistent at that time to compare all germicides to phenol, since this was, and still is, a widely known antiseptic of very constant properties. The result has been that all kinds of germicides have been compared to phenol and their relative value expressed as a "phenol coefficient." While this test has served its purpose admirably during these twenty and more years, it has all this time been misleading in one particular, that the ability to kill the test organism used, B. typhosus, does not necessarily mean that other pathogenic bacteria will also be killed by the germicides tested in this way. There is much information available now to show that such is not the case.

Disinfectants and antiseptics are used to kill pathogenic microorganisms and not simply for killing B. typhosus. Tilley[4] has stated that " . . . a phenol coefficient has no practical value whatever, except as a means of preventing the use of positively worthless preparations and as an aid to the manufacturer in maintaining uniformity of his product." This is in keeping with the present trend of recognizing the "specificity" of germicidal action of disinfectants and antiseptics. I[5, 6, 7] have approached the subject in a somewhat more positive manner and have suggested definite organisms, representative of the main group of pathogens, to be used in a test for determining the bactericidal efficiency of germicides. According to this scheme, phenol coefficients could still be used by the manufacturer for controlling the uniformity of his products, but the dilutions of these products which are specified for actual use must be based on definite tests against the organisms it is desired to kill. Antiseptics for use on the skin and mucous membranes and in cuts, abrasions, wounds, etc., should be tested against pyogenic organisms, such as Staphylococcus aureus and Streptococcus hemolyticus. If a disinfectant is recommended for general use, the manufacturer must be sure that the dilutions specified for his product will kill all of the non-

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sporing organisms which cause diseases of an epidemiologic nature. If he so desires he may recommend only one dilution for general use, but he must be certain by actual tests that this dilution will kill those organisms that are dangerous from an epidemiologic standpoint. [5, 6, 7] have already established standards for these representative test organisms, so that the laboratory worker can be sure that he is working with cultures of normal vigor and resistance. I have also outlined methods for testing all liquid germicidal substances from which aqueous solutions can be made. It will not, however, be possible here to discuss these methods further.

We are interested here only in discussing methods of testing antiseptics. There is at this time a certain amount of confusion with respect to the meaning of the word "antiseptic." Like so many other English words, "antiseptic" has two meanings according to the way in which it is used. When referring to an antiseptic for use in preserving food or any animal or vegetable material, we have in mind a substance which will prevent growth of the microorganisms which cause food spoilage. The original meaning of the word is, literally, "against putrefaction" and until about 1865 this term was used to indicate only food preservatives. Since the time of Lister's epochal work the term has taken on an additional meaning. Germicides advocated by him for use in antiseptic surgery were called "antiseptics." From this time on the dictionaries have given two meanings for this word: (1) substances which will prevent putrefaction and fermentation in animal and vegetable matter and (2) substances used to destroy pathogenic microorganisms. In a recent paper by myself[8] the following definition was given: "Antiseptics are substances which when applied to microorganisms will render them innocuous either by actually killing them or preventing their growth according to the character of the preparation or the method of application." This matter is discussed fully by me in an editorial in the *Journal of American Pharmaceutical Association*.[9] The medical profession has used the term as synonymous with "germicide" and when they use antiseptics in a practical way they expect them to kill pathogenic microorganisms.

There are many types of antiseptic preparations and methods of application by means of which infective organisms are merely held in check and not actually killed by the chemical agent used. The use of wet dressings, irrigations, such as the hypochlorite method of treating war wounds advocated by Carrel and Daken, the use of bacteriostatic dyes, the proper application of antiseptic ointments and salves, etc., will, when properly applied, be effective in preventing the activity of microorganisms and leave them easy prey to the leucocytes. When testing the efficiency of such preparations, methods must be used which are quite different from that employed for the ordinary liquid antiseptics.

METHOD OF TESTING LIQUID ANTISEPTICS

In the original Rideal-Walker method[1] for obtaining phenol coefficients 5 drops of culture were added to 5 c.c. of germicide for two and one-half to fifteen minutes at 15° to 18° C. The present Rideal-Walker Test,[1] 1921 revision, calls for 0.5 c.c. of culture of B. typhosus to 5 c.c. of diluted germicide, while in the Hygienic Laboratory test 0.1 c.c. of culture is added to 5 c.c. of

germicide. After years of experience with these methods and comparative tests with representative germicides, we find that the use of 0.5 c.c. of culture to 5 c.c. of diluted germicide without the addition of other organic matter is preferable for many reasons and, furthermore, it is simpler to add organic matter in the form of culture than to add it separately. Since liquid antiseptics as ordinarily used on the human and animal body are intended to prevent infection by killing pathogenic microorganisms, especially the pyogenic group, or to aid in curing such infections if they have already started, the organisms used for testing their suitability for this purpose should be the ones which are actually met with in practice. It is obvious that the ability of an antiseptic to kill B. typhosus, the test organism used in our standard phenol coefficient tests, is of little value in determining its germicidal efficiency against the organisms causing infections. A phenol coefficient of an antiseptic is naturally of little value to the medical profession and the layman in making a choice from the large number of germicides available. There are substances which are effective against B. typhosus but which will not kill Staphylococcus aureus at all. There are other germicides which kill Staphylococcus aureus in high dilution and will not kill B. typhosus except in concentrated solution. Since the vast majority of skin and wound infections are caused by Staphylococcus aureus and since this is the most resistant of the pyogenic organisms, it has been selected as a test organism for determining the germicidal efficiency of liquid antiseptics.[5] It has been found by experience that the dilution of germicide which will kill Staphylococcus aureus will also kill the streptococci. The method given below is a test of those antiseptics which are used on short time application, that is, where they are not kept in contact with infected surfaces for a long period of time. The method used for this purpose is briefly as follows:

Staphylococcus aureus is grown in nutrient broth composed of 0.5 per cent Liebig beef extract, 1 per cent Armour's peptone, and 0.5 per cent sodium chloride in distilled water, adjusted to P_H 6.6 to 6.8. After three successive transfers in this broth at twenty-four-hour intervals, incubated at 37° C., 0.5 c.c. of this broth culture is added to 5 c.c. of the undiluted or diluted antiseptic in large tubes which are held at 37° C. At intervals of five, ten, and fifteen minutes, transfers are made from this antiseptic culture mixture to sterile tubes of broth of the above composition and incubated at 37° C. for forty-eight hours. The tubes are then observed for evidence of growth. The dilution of the antiseptic which is capable of killing these test organisms in five minutes is considered to be efficacious when used in practice. The preparations which are tested by this method are the ordinary antiseptics for use on the skin, for cuts, wounds, mucous membranes, etc., and mouth washes which claim antiseptic properties. In this test, the organic matter is present in the form of broth and culture. This is more convenient and less expensive than the practice of adding organic matter separately. The test being carried out at body temperature gives the antiseptic the benefit of greater activity at the higher temperature and is much fairer to the preparation being tested than is the room temperature quite generally used in the past.

Those preparations which are sold in tablet or powder form from which antiseptic solutions are made by the addition of water are examined by the test outlined above.

ANTISEPTIC OIL PREPARATIONS

Many antiseptic preparations have the active ingredient incorporated in some inert mineral oil. Regardless of the nature of the oil base which carries the active ingredient, the test as outlined above would not be applicable. Instead a method which we call the "Filter Paper Method" is used. This test is, briefly, as follows:

No. 2 Whatman Filter paper is cut into pieces about 0.5 cm. square, placed into a test tube, plugged with cotton and sterilized in the hot air oven at not over 170° C. (to prevent charring). The desired number of these sterile squares are then immersed in a twenty-four-hour broth culture of the test organism, Staphylococcus aureus. (This culture must be a fresh resistant strain which is not killed by a 1-70 phenol in ten minutes, nor by 1-80 phenol in fifteen minutes at 20° C., following the method given above.) These paper squares, impregnated with the culture of Staphylococcus aureus, are then fished out with a sterile culture wire (bent on the end) and transferred to a tube of broth (10 c.c.) of the above composition. By shaking thoroughly at intervals over a period of five to ten minutes, the excess of the antiseptic oil is washed off from the paper squares. The pieces of paper are then fished out and transferred to another tube of broth (10 c.c.) and incubated at 37° C. for forty-eight hours. The tubes are then observed for growth.

Some of the antiseptic oil preparations are recommended for use in such a way that only short time application is involved. If they are recommended for use in surgical dressing where contact with the infective organisms is assured over a long period of time, an inhibitory test would then be applicable. (See method for antiseptic dyes.)

POWDERS

Under this heading are included all powder preparations which claim to be antiseptic and which are applied directly to the skin, mucous membranes, and infected surfaces. Those powders which are used in such a way that no conditions are established whereby they are kept in continued contact with the infected surface, such as talcum powders, tooth powder, etc., are examined by the "Filter Paper Method" as follows:

Sterile filter paper squares impregnated with Staphylococcus aureus as outlined above, are covered on both sides with the powder and allowed to remain in this intimate contact for five minutes. At the end of this time period the piece of paper is fished out and transferred to a tube of broth and, after the adhering powder is shaken off, again transferred to broth and incubated at 37° C. for forty-eight hours. These powders which are recommended for dusting into infected wounds may be examined as outlined later.

ANTISEPTIC TOOTH PASTES AND SHAVING CREAM

In the examination of these two types of preparations, we are faced with the necessity of determining their efficiency in the undiluted state because it is practically impossible to fix a definite dilution of each product which will simulate the dilution of the preparation when used in practice. We have, therefore, seen fit to test the antiseptic properties of these preparations undiluted. Contact with infective organisms is very short at best, and a test to determine killing effect is applicable. The "Filter Paper Test" is used for this purpose and the procedures as outlined above are followed, Staphylococcus aureus being the test organism. In this case the impregnated pieces of paper are completely covered with the paste and this contact allowed to continue for five minutes at 37° C.

Because of the fact that some dentifrices may be more effective in solution than in the paste form, a 50 per cent solution is also made and tested by the filter paper method in the same manner as indicated for the undiluted paste. The active ingredients have a better opportunity of coming into contact with the bacterial cells when in a solution. If the tooth paste or shaving cream kills Staphylococcus aureus either in the undiluted form or in a 50 per cent solution (1—1) in five minutes at 37° C., it will be considered as an antiseptic preparation.

ANTISEPTIC LOZENGES AND ANTISEPTIC SUPPOSITORIES

Lozenges and suppositories for which antiseptic claims are made are examined in a manner which approximates to a great extent the conditions met with in practice. These preparations dissolve slowly giving a solution of their active ingredients in the local secretions. There is no way of accurately determining the concentration of solution obtained in the practical use of such products. For this reason we simply test these preparations in a concentrated aqueous solution. In this case more benefit is given the product than is probably justified, but considering all the factors involved this is the fairest method of testing. The filter paper method is then used as follows: pieces of paper impregnated with Staphylococcus aureus are allowed to remain in a saturated aqueous solution of the product for as long a time as is required for the preparation to dissolve when used as directed. For the antiseptic lozenges this requires about ten to fifteen minutes. Some suppositories require a longer time and the period of the test is regulated by the time it takes for solution in distilled water. The usual procedure for the filter paper test is followed.

ANTISEPTIC SOAPS

For the testing of antiseptic soaps the filter paper method is applicable. In fact filter paper simulates fairly closely the conditions found in the skin. In the case of liquid soaps, the undiluted soap is used in the filter paper test as outlined above. If the soap is in powder or cake form, a thick lather is made aseptically in sterile water in a sterile Petri dish, and the filter paper test carried out in the usual manner.

ANTISEPTIC SALVES, OINTMENTS, BUNION PADS, CORN PLASTERS, SURGICAL DRESSINGS AND POWDERS FOR WOUND DRESSINGS

When such preparations as these are used in practice, the active ingredients are held in intimate contact with the infective microorganisms and can render them innocuous by simply preventing their activity. Since these preparations do remain in contact with the infected surfaces for long periods of time when used in practice, laboratory tests for their efficiency should simulate these conditions as nearly as possible. It is evident that such tests as have been outlined above would not be applicable; we are not interested here in killing organisms within a certain length of time. If an antiseptic salve, for example, does prevent the growth of microorganisms in an infected area, it will not only prevent them from doing harm, but will also render them easy prey for the leucocytes. For this reason, the following method is applicable for this type of product:

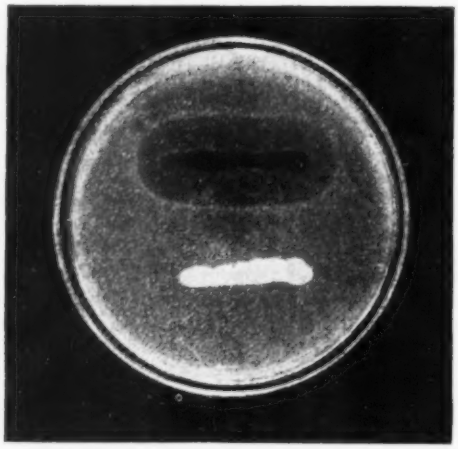

Fig.* 1.—Illustrating the activity of an antiseptic ointment against Staphylococcus aureus. The dark streak is antiseptic ointment which is surrounded by a clear zone in which no organisms have grown. The white streak is control ointment without antiseptic. The surrounding turbidity is a heavy growth of Staphylococcus aureus.

Staphylococcus aureus of normal resistance[5] is grown at 37° C. in the broth described above and transferred in this medium for three consecutive days. One-tenth of a c.c. of a 1-100 dilution of this culture is added to 15 c.c. of melted 10 per cent serum agar (1.5 c.c. sterile normal horse serum added to 15 c.c. of nutrient agar [1.5 per cent] made from the above broth base) at 45° C., the culture thoroughly mixed in the serum-agar and poured into a sterile Petri dish and allowed to cool at room temperature. As soon as this inoculated agar has hardened, the salves and ointments, previously melted at 37° C., are streaked over a small surface of the inoculated agar with a sterile glass rod. Melted sterile vaseline is spread on another part of the inoculated agar in the same manner and the plate, inverted, incubated at 37° C. for forty-eight hours. After being incubated it will be noted that colonies of Staphylococcus aureus grow immediately adjacent to the vaseline

*In all of these illustrations mercurochrome was used to illustrate the methods discussed.

control and even under it. There is no active ingredient in pure vaseline which will prevent the growth of Staphylococcus aureus. However, in effective antiseptic salves and ointments a part of the active ingredients contained in them is absorbed into the serum-agar, which is about one-eighth of an inch deep, and by their presence prevent the organism present from growing (see Fig. 1).

The plate will show a clear zone around the antiseptic salve or ointment which is in marked contrast to the turbidity of the surrounding medium caused by the heavy growth of the organism. In treating infected surfaces with preparations of this nature, it is necessary that the active ingredients leave the inert base and become free to surround the infective organisms. It is only in this way that the preparation will be of benefit in preventing the growth of or killing these microorganisms. If the antiseptic were so

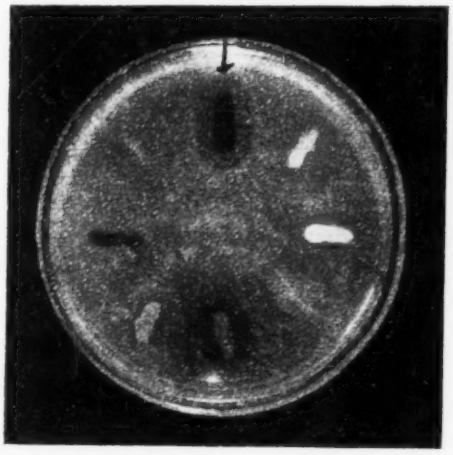

Fig. 2.—This is a comparison of eight so-called antiseptic ointments obtained from the retail market. Five of these have absolutely no effect on Staphylococcus aureus, one slight effect and two show a definite zone of sterilization.

securely incorporated in the inactive base that it could not become free to attack the microorganisms or if the base were of such a nature that the antiseptic could not separate from it, the value of such preparations so far as the antiseptic ingredient is concerned would be lost. Serum-agar simulates fairly closely the conditions met with in skin and wounds.* It is permeable, semisolid, isotonic, contains a body fluid, and constitutes a laboratory means of approximating the conditions found in human and animal tissues, at least so far as the preparations under consideration are concerned. The clear zone may indicate germicidal action and not just bacteriostatic effect. To prove this, small pieces of agar from various parts of this zone are inoculated into broth and incubated for forty-eight hours. When the active ingredient comes out in sufficient concentration to be germicidal in the clear zone this is of even more interest because it indicates greater practical value of the preparation.

*The addition of normal serum to 1.5 per cent nutrient agar gives a medium which closely simulates practical conditions.

As a matter of interest a number of widely advertised so-called antiseptic ointments which at this time enjoy a very wide sale were tested on a Staphylococcus aureus poured plate (see Fig. 2). It will be noted that six of these ointments have no effect whatever against staphylococcus aureus while two of them give a definite zone of sterilization. Antiseptic ointments should certainly kill or inhibit Staphylococcus aureus. In this test if the active ingredients will leave the ointment and go into the surrounding medium they can inhibit Staphylococcus aureus and cause a clear zone, but these six ointments do not even do this. It can be safely said that they will not be effective when used in practice.

In testing bunion pads, corn plasters, adhesive, surgical dressings, and powders for wound dressings for which antiseptic claims are made, the procedure just given is employed. These preparations are simply placed on top of a poured serum-agar plate containing Staphylococcus aureus, and incubated

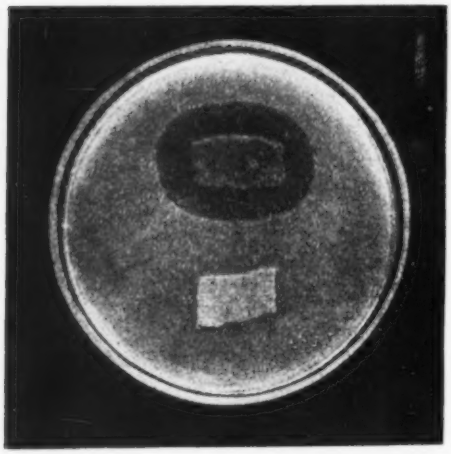

Fig. 3.—This illustrates the bactericidal efficiency of an antiseptic gauze as compared to gauze which is supposed to contain an antiseptic. The wide zone around the upper antiseptic gauze shows that the active ingredient has passed out into the surrounding medium.

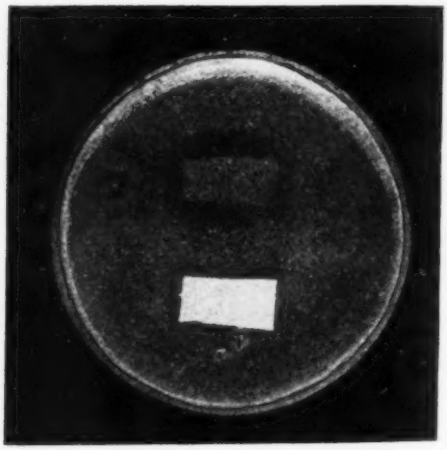

Fig. 4.—This shows an antiseptic adhesive as compared with ordinary adhesive, the dark strip above being that which contains the antiseptic. These are placed on the surface of the medium.

at 37° C. for forty-eight hours. The antiseptic in such products permeates the serum-agar medium and prevents the growth of the test organism (or actual killing may take place). The amount of antiseptic contained and its solubility, together with the ease with which it leaves the base, are factors in determining the width of the clear zone around these preparations (see Figs. 3 and 4).

This same serum-agar plate method can be used for testing antiseptic catgut. In this case pieces of the catgut are imbedded into the shake serum-agar culture of Staphylococcus aureus after it has been poured into a plate and cooled; it is then incubated at 37° C. (see Fig. 5). Fig. 6 illustrates the relative merits of five different antiseptic catguts obtained from the retail market. It is to be noted that number three is without any effect on Staphylococcus aureus, whereas Nos. 1 and 4 are of slight effect, while Nos. 2 and 5 give a wide zone. This is about the only way this type of preparation can be tested.

ANTISEPTIC DYES

In examining antiseptic dye solutions we must consider that we are dealing with a liquid antiseptic whose action is supposed to continue for a long time. When these dyes are applied to infected surfaces or wounds they are expected to penetrate tissue and continue their activity. Because these dyes remain

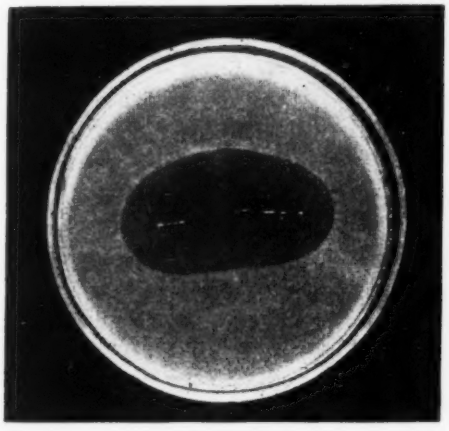

Fig. 5.—In this case antiseptic catgut is imbedded in the agar. Penetration of 1.6 cm. is shown, as well as to a depth of one-eighth of an inch.

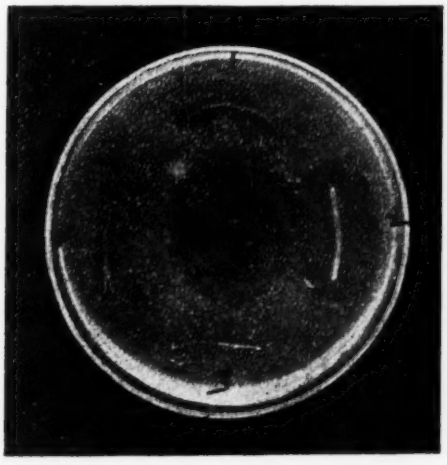

Fig. 6.—Comparing the efficiency of five different antiseptic catguts obtained from the retail market. No. 3 has no bactericidal effect against Staphylococcus aureus, Nos. 1 and 4 slight effect, whereas Nos. 2 and 5 have considerable penetration and bactericidal action.

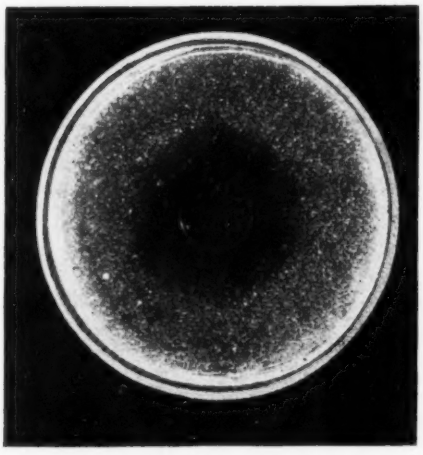

Fig. 7.—This illustrates the penetration and antiseptic activity of a liquid antiseptic dye. This antiseptic penetrates the medium for 1 cm. from the edge of the cup.

for an indefinite time in the tissue to which they are applied the test which simulates this condition must also be used. For this purpose the serum-agar Cup-Plate Method is applicable.* (see Fig. 7). The proper amount of staphylo-

*I am indebted to Dr. L. C. Himebaugh of the Pease Laboratory, of New York City, for this method.

coccus aureus culture is added to 15 c.c. melted serum-agar cooled to 45° C. (as described above) and poured into a sterile Petri dish. A sterilized glass stopper 1.5 cm. in diameter is then placed in the center of the dish and left until the agar has hardened. The glass stopper is then removed by twisting slightly and the small cracks and crevices are sealed with about 3 drops of sterile agar. The antiseptic dye solution to be tested is placed (0.25 c.c.) into this cup-like depression and the plate covered with an unglazed clay top. This is then incubated at 37° for twenty-four to seventy-two hours. If the antiseptic penetrates the medium in germicidal or bacteriostatic strength there will be a clear zone around the cup (see Fig. 7). Subcultures into broth from different parts of the zone are necessary to determine whether or not the substance is germicidal or merely bacteriostatic. This method is of value also in testing the ability of antiseptics to retain their strength over a long period of time. One of the common antiseptics* in use today was so tested after five years in solution and gave a zone equal to that formed by a fresh solution. It is also useful for testing the penetrating power of any liquid antiseptic.

SUMMARY

Methods for testing the efficiency of various kinds of antiseptic preparations are briefly given. Laboratory tests cannot, of course, simulate the exact conditions found in practice, but the procedures outlined here are an attempt to do this and apparently they do approximate these conditions to a very marked degree. More complicated methods would be expensive, involved and time-consuming, and would probably introduce factors which would make the tests more difficult to duplicate. If substances prove to be antiseptic by the tests outlined here, there is every reason to believe they will be efficacious when used in practice.

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*One per cent solution of mercurochrome.

A SOURCE OF INCONSTANT ERROR IN THE DARE HEMOGLOBINOMETER*

By Frank G. Haughwout, Manila, P. I.

CLINICAL pathologists, physicians, and others to whom falls the task of making routine hemoglobin estimations, have long realized the inaccuracies inherent to the so-called "rapid clinical methods." The Newcomer disc method and the procedures of Cohn and Smith, and of Palmer, all yield results of far greater accuracy than the apparatus of Tallqvist, Sahli, Dare and others, but for the most part they are impracticable in the office of the average physician, and are so considered by many laboratories of some pretension.

The instrument introduced in 1900 by Dare[1] is based on sound principles and is so eminently practical in its construction and mode of use that its adoption has become widespread until it now is looked upon as the most accurate of the instruments for rapid hemoglobin determination. It, accordingly, has been adopted by a considerable number of large institutions. However, the instrument has defects which, to me, seem to be due more to carelessness in its manufacture than to inherent errors in principle.

Senty[2] in a comparative study of different methods of hemoglobin determination has shown that the instrument reads too low in the upper part of the scale, that is to say, above 65 per cent. He also has shown that these errors are, to a large degree, modified by grinding off 15 per cent of the upper end of the scale as suggested by Sanford.

I discovered another source of error lying in the pipettes into which the blood is drawn. As I have seen no mention of this fallacy in the current literature, I am putting it out as a warning to other workers who may not be aware of the possibilities for error in their own instruments. The discovery came about in this way.

An order had been placed through a local importing house for a Dare hemoglobinometer. Two extra pipettes also were ordered to provide against the contingency of breakage. The two extra pipettes were delivered several weeks before the instrument and its accompanying pipette. The three pipettes were placed in service to be used interchangeably with the instrument, it being assumed that they were standardized. Unfortunately, it is not possible to say which of them was delivered with the instrument. It is not essential to the purposes of this discussion whether any one of them was accurate. The main point is that no two of them agreed within even 8 per cent. Prior to the delivery of the Dare instrument hemoglobin estimations had been made with the Tallqvist scale, the standard directions for its use being closely followed.

Several weeks before the delivery of the Dare instrument I had, at the

*From the Laboratory of Biology, Pathological Society of Manila, P. I.
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instance of Dr. H. E. Stafford, of Manila, undertaken the study of the bowel discharges of a local business man. In the course of the investigation it seemed desirable to examine the blood, so erythrocyte counts and hemoglobin determinations were made at intervals, the latter being made with the Tallqvist scale. The hemoglobin findings were as follows:

January 27	75 per cent
March 3	70 per cent
March 26	75 per cent

At this point the patient went up into the hills where he remained until April 17. A few days later he came to the laboratory. It was found that his erythrocyte count (taken with a Trenner pipette each time) had risen from 4,670,000 to 5,220,000. The Dare instrument having arrived, his hemoglobin was taken by it and was found to be 64 per cent. This was startling, but the colors appeared to match perfectly so the matter was reported to Dr. Stafford. A few days later the patient went to another laboratory for a Wassermann test. There he spoke of the apparent fall in his hemoglobin whereupon the physician there examined his blood with another Dare instrument and told the patient that his hemoglobin was "nearer 90 per cent than 60 per cent." The patient naturally reported this to his physician who in turn communicated it to me.

On learning this I communicated with the physician who had made the second determination and suggested the desirability of a comparison of the two instruments. He, on his part, contended that the instruments were standardized at the factory and it was unlikely that a discrepancy could exist between them. His lack of interest in the matter led to the dropping of that part of the investigation.

At my request, Dr. Stafford sent the patient to the laboratory on April 23. His hemoglobin was read again by the Dare instrument, both by me and by his associate, Mr. John K. Pickering, of the biologic staff of the Pathologic Society. I made a reading of 76 per cent while Mr. Pickering read 73 per cent. In each of these instances (April 17 and 23), pipettes were taken from their boxes at random, so it is impossible to say which of the three was used in either instance. The obvious discrepancies in these two readings seemed to indicate the necessity for an inquiry into the reasons thereof.

Mr. Pickering kindly volunteering to furnish the blood and to assist in a series of experiments, an investigation was started without, however, any definite idea as to just where the trouble lay. A number of readings were taken on different days, the pipettes being taken at random, with the result that conflicting and irreconcilable percentages were obtained. On going over the figures, however, a marked grouping was noted and ultimately it was found possible to arrange the readings in two groups which, for convenience, may be designated as the 58 to 60 per cent and the 70 per cent groups. This gave a clue to the situation.

The pipettes were separated and designated A, B, and C respectively, and readings made by them were so classified. Each pipette was read four times, twice by Mr. Pickering and twice by myself, on each of four separate days, each observer accordingly, having read each pipette eight times. In every

case the blood was taken from Mr. Pickering. The readings were consistent throughout, showing only the minor variations customarily observed. All readings were by candle-light in a closet. The results are summarized in the averages shown in Table I.

TABLE I

PIPETTE	PER CENT HEMOGLOBIN	
	HAUGHWOUT	PICKERING
A	75⅛	73½
B	58¾	58⅞
C	82¾	82¼

This made it apparent that there was a constant difference of more than 20 per cent between the readings with Pipettes B and C on identical blood. It seemed obvious that the capillary chambers must be of unequal depths.

To ascertain if this were so, measurements were made with the ocular micrometer on a compound microscope, of the distance separating the two glass elements forming the capillary chamber, that is to say, the depth of the

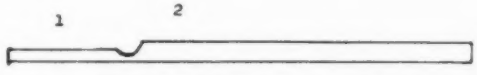

Fig. 1.—Points of measurement of capillary pipette.

chamber. The glass plates were clamped in the regular holder and the objective was focused down on the inner edges and the distance separating them measured. The measurements obtained are shown in Table II.

TABLE II

PIPETTE	DEPTH MILLIMETERS
A	0.175
B	0.140
C	0.192

These measurements are seen to be consistent if one compares them with the hemoglobin readings given in Table I. However, the beveling of the glass made correct focusing rather difficult, thus creating a possible source of error, so it was decided to repeat the measurements using a micrometer caliper. Unfortunately one reading in the metric system could not be obtained, but a Starret instrument measuring in thousandths of an inch was kindly loaned by the Atlantic Gulf and Pacific Company of Manila. The white glass element of each pipette was measured at the points indicated in the diagram composing Fig. 1.

The values obtained by these measurements are set forth in Table III.

TABLE III

PIPETTE	1	2	DEPTH OF CHAMBER
A	91.5	98.5	7.0
B	86.0	92.5	6.5
C	85.0	92.5	7.5

While no claim is made for absolute accuracy in these measurements it will be seen that they run parallel with the hemoglobin readings.

To test the matter, eight readings (four by each observer) were made on the same day with Pipette B. The first four were made with the pipette as it stood, and an average reading of 59¼ per cent was obtained. Then, a slip of paper measuring 0.003 inch in thickness was clamped between the two glasses and the pipette again filled. This time it gave readings averaging 92 per cent.

DISCUSSION

Dare lays stress on accuracy in the depth of the capillary chamber although he does not say what this should be.* He states that the depressed surface of the white glass is ground and "measured with a micrometer to give an exact depth." He adds that when clamped, the chamber "contains a blood-film of definite thickness," and finally, "The pipettes all contain a blood stratum of standard thickness, and can therefore be used with any instrument, a distinct advantage over the calibrated pipettes which have a capacity only adapted to one particular apparatus."

It is very evident that the pipettes that form the subject of this discussion do not conform to these specifications, and it is an entirely legitimate subject for disquieting speculation as to just how many similarly inaccurate pipettes are distributed about in various laboratories and in daily use. So long as one pipette is continuously used with a given instrument and substitution by another is made only when the first is broken, the readings will be relatively consistent, be they accurate or inaccurate. Unless the same group of patients is under study, the eventual substitution of another pipette that may be read either higher or lower than the first may not be followed by a series of readings that of themselves would lead to lurking doubts in the mind of the user. Unless some one else has occasion to check his reading on the blood of some particular person, as happened in the instance recorded in this paper, the hematologist may continue to use an inaccurate pipette indefinitely, serenely unconscious that his determinations are inaccurate, or that the pipette he is using today may differ in its readings by 10 or even 20 per cent from the one he used a few months ago.

The three pipettes composing the set in my possession presented a definitely graded set of readings. Selected at random they might either show a progressive improvement or a progressive deterioration in the condition of the blood. On the other hand, they might yield a set of irrational and widely divergent readings that in the end would most assuredly destroy the faith of the clinical man in his hematologist.

CONCLUSIONS

1. Senty already has indicated the fallacies that lie in the upper segments of the comparison prism of the Dare hemoglobinometer. These errors are constant and may be largely compensated by grinding off a portion of the upper segment of the prism.

*Senty says it is "about 0.18 mm."

2. It has been shown in this paper that inaccurate grinding of the capillary chambers of the blood pipettes leads to the production of chambers of unequal depths that accordingly yield inaccurate values with the blood sample. These errors, of course, are inconstant and their extent cannot be known to the user of the instrument. Grave errors in hemoglobin determination naturally follow and these cannot fail to exert a profound influence on treatment and prognosis, to say nothing of diagnosis.

3. Both sources of error may be avoided by greater attention on the part of the manufacturers to the original specifications for the construction of the instrument as set forth by Dare in his original paper.

4. All Dare instruments at present in use should be checked as to color prism. More important, however, than this is the standardization of every pipette with a view to discover if the chamber is of standard depth to harmonize with the values expressed by the comparison prism.

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A TECHNIC FOR STAINING RED BLOOD CELLS FOR MEASUREMENT OF CELL DIAMETERS BY THE PROJECTION METHOD*

By Francis P. Parker, B.S., Emory University, Ga.

FIX smear by ordinary heat method. Apply a saturated solution of eosin in 95 per cent alcohol for two minutes and wash with distilled water and drain. Next, flood with a 1 per cent water solution of methyl violet. This is left on for two minutes, washed with distilled water and the slide dried over a flame.

The above procedure stains the red cells a deep bluish violet, and the white cells a light violet with deep nuclei. In the measurement of red cell diameters it is necessary to have sharp contrast between the cell margin and background and this stain supplies this feature, accentuating the cell outline much more than Wright's or other stains common in blood examination.

It is essential that the order given above be carried out due to the action of alcohol in preparing the cell for the reception of the water soluble methyl violet. Otherwise best results are not obtained. The heat fixation method proved preferable in simplicity and shorter staining time to both the methyl alcohol and absolute alcohol methods.

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MICROSCOPIC PROJECTIONS IN THE MEASUREMENT OF ERYTHROCYTES*

By Francis P. Parker, B.S., George T. Lewis, Ph.D., and J. L. McGhee, Ph.D., Emory University, Ga.

THE use of the microscope as a projection apparatus has long been in practice in embryologic work where no special attention was given to the exact magnification of the projected image. To achieve this an ordinary monocular microscope of the type generally used by medical students is employed. It is broken at the inclination point so that the long axis of the body tube is at right angles to the pillar. The draw-tube and collar in which it slides is removed from the body-tube. The upper planoconvex lens of the Abbe condenser and the mirror are also removed. This arrangement permits the scope to be fixed in a position with both the base and the tube perpendicular to the horizontal, and the source of illumination immediately above the condenser. Where large magnification is desired, the intensity of illumination must be great. In this application a 250 watt flood light with concave reflector and planoconvex lens, three inches in diameter, was used. Both the scope and light box are fixed with set screws on a metal bar set upright in a 20 inch by 20 inch, two inch oak base, heavy enough to act as an anchor for the apparatus. The image is projected on a sheet of white paper thumb-tacked to the base.

In using this apparatus for measuring blood cell diameters, the distance between the surface of the stage and the surface of the base must be exactly 36 inches. When the Leitz $\frac{1}{12}$ inch, 1.8 mm., objective, oil-immersion lens is used, this distance gives a magnification of 500 diameters. Therefore, the projected red cell is measured in two directions in millimeters, and the sum of the two measurements is the red cell diameter in microns.

With this method it is possible to take cell measurements to 0.25 microns. The conditions governing the selection of cells to be measured and the number of cell measurements required for a diagnosis are the same as those already set forth.

The magnification may be conveniently checked from time to time by use of the standard Levy counting chamber. The ruled squares are $\frac{1}{400}$ mm. in area so when projected at 500 diameters they should measure 25 mm. across. The chamber is reversed to discount error due to thickness of the plate.

ADDENDUM

Since the above was written, we have adopted a 400 watt flood light bulb as the source of illumination. This gives a brighter field and less eye strain when a series of determinations are to be made. We have also attempted to increase the magnification by means of a projection ocular, but the amount of light is cut down to such an extent as to prohibit its use.

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THE HINTON GLYCEROL-CHOLESTEROL PRECIPITATION TEST FOR SYPHILIS

A Comparative Study With the Wassermann Test in 2120 Cases*

By Edgar J. Munter, M.D., Boston

THE Wassermann reaction long has been the standard serum test for syphilis, in spite of the fact that it is not infallible and in spite of its technical difficulties. Many new tests for syphilis of greater simplicity have been described, but have not proved to have greater delicacy or accuracy. In June, 1927, Hinton described a new, simple, easily read, sensitive test for syphilis which required less labor, apparatus, and material than the Wassermann reaction, which had fewer false positives, and which was more sensitive than either the Wassermann or Kahn reaction. This report consists of an analysis of 2331 consecutive Wassermann and Hinton glycerol-cholesterol reactions on 2120 patients in the Peter Bent Brigham Hospital and its out-patient department between the months of November, 1927 and April, 1928.

The laboratory tests upon which this paper is based were performed as follows:

1. *Wassermann Reaction.*—The technic is that published by Hinton.[1]

Two antigens were used separately, one a simple, cholesterolized, alcoholic extract of guinea pig heart, and the other an alcoholic extract of human heart, half saturated with cholesterol at 17° C. The hemolytic system consisted of 2 units of amboceptor, 2 units of complement, and 0.5 c.c. of a 5 per cent suspension of sheep's corpuscles. One-tenth cubic centimeter of serum was used for each antigen, and 0.2 of a c.c. for the anticomplementary control. The primary incubation was for forty minutes at 37° C. and the secondary incubation for one hour at the same temperature. Tests were read immediately after the secondary incubation. They were reported as positive, negative, and doubtful.

2. *Glycerol-Cholesterol Precipitation Reaction.*—The glycerol-cholesterol precipitation reaction, which Hinton believes is, in reality, not a precipitation test, but an agglutination reaction, was executed as described in his article in the *Boston Medical and Surgical Journal*,[2] with the following modifications which are soon to be published in detail by Hinton.

(a) His stock indicator consisted of the ether-insoluble, alcohol-soluble fraction of beefsteak muscle which he added to a 0.7 per cent solution of cholesterol in absolute alcohol, in the proportion of one part of the extract to nine parts of the cholesterol solution. (b) The temperature of incubation was 37° C. rather than 27° C. (c) The amounts of serum used were 0.1, 0.2, 0.3, 0.4, and 0.5 of a c.c. (d) The salt concentration varied in proportion to the amount of serum used, as given below.

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Briefly, the technic was as follows: Glycerinated indicator was prepared by adding 2 parts of salt solution for 1 part of his stock indicator, and shaking vigorously for three to four minutes. Five per cent salt solution was used for diluting his stock indicator for the 0.1 and 0.2 c.c. amounts of serum, 3 per cent salt was used for the 0.3 and 0.4 c.c. amounts, and 2 per cent salt for the 0.5 c.c. amount. Further dilution was made by adding 12 parts of salt solution, shaking, and finally adding 15 parts of 50 per cent glycerol solution in distilled water. The amounts of serum were pipetted into serum tubes of about 10 mm. diameter, and to each was added 0.5 c.c. of glycerinated indicator. The tubes were shaken for two minutes and placed in the incubator at 37° C. for a period of sixteen hours, at the end of which time the tests were read. A slight clearing, which sometimes occurred in any or all tubes, was regarded as negative. Positive reactions were reported when any or all of the tubes showed complete clearing of the suspension, with a resulting water-clear fluid in which *large flocculi*, consisting of the agglutinated particles of cholesterol, were suspended. When there were no flocculi, the test was reported as negative. These reactions are very clear cut and are much more easily read than is the Kahn test.

We found that the Hinton and Wassermann tests agreed absolutely 2122 times in the total of 2331 tests, an absolute agreement of 90.6 per cent, or, speaking in terms of patients rather than tests, there was agreement in 1975 cases in the total of 2120 (93.4 per cent agreement). Of the 6.6 per cent, or 145 cases, in which the tests gave different results, we found that the Wassermann result agreed with the clinical findings in 41 instances, while the Hinton test coincided in 82, and that there were 22 cases in which the correctness of the reaction could not be proved. Thus, in the small percentage of cases where the tests failed to agree, the result of the Hinton test coincided with the clinical findings just twice as many times as the Wassermann test.

TABLE I

Wassermann / Hinton	+ / +	− / −	± / ±	− / +	+ / −	± / +	± / −	− / ±	TOTAL	PER CENT AGREEMENT
Number of cases	145	1825	9	41	13	23	36	28	2120	93.4
Number of tests	195	1920	9	54	25	48	51	29	2331	90.6

The cases were divided as follows: Group I, 1883 cases in which no clinical evidence of syphilis could be obtained; Group II, 215 cases of clinical syphilis as determined by a positive history or physical examination; Group III, 22 cases in which the clinical evidence was insufficient to exclude syphilis and who could not be gotten back for follow up, classed as doubtful cases.

In the 1883 nonsyphilitics, the Wassermann was negative 1847 times, or in 92.7 per cent; and the Hinton 1851 times, or in 92.8 per cent. The Wassermann was positive 11 times, as compared to 7 positive Hinton tests, and both tests were doubtful in 25 cases. There is, then, very little difference in this group in the results of the two reactions, the only variation being a few less false positive Hinton tests.

In the group of clinical syphilis, however, the results are more striking. The Wassermann was positive in only 148 out of the 215, while the Hinton test was positive in 184. Eleven of the 215 cases might be considered as cured or

latent cases and legitimate misses for both tests, for these 11 were all early cases of syphilis which had been treated intensively and had been serologically negative for some time. This leaves us with the conclusion that the Wassermann test was positive in 72 per cent of the cases of clinical syphilis (treated and untreated), while the Hinton test was positive in 89.7 per cent, and that there were 26 cases of syphilis which the Wassermann would miss entirely, but only 10 which would have been missed by the Hinton test.

The Wassermann reaction was doubtful 30 times in the presence of syphilis and 25 times in nonsyphilities, whereas the Hinton test was doubtful in 11 syphilities and in 25 nonsyphilities: in this series a doubtful Wassermann had just about an even chance of occurring with syphilis, while a doubtful Hinton test two out of three times occurred in nonluetics.

TABLE II

	WASS. +	HINTON +	WASS. −	HINTON −	WASS. ±	HINTON ±
Cases of Syphilis	148	184	37	21	30	11
Nonsyphilitic Cases	11	7	1847	1851	25	25
Doubtful Cases	1	18	14	2	7	2

Both the Wassermann and Hinton tests were positive in 145 cases. One hundred and forty of this group were cases of proved syphilis, and the remaining 5 were cases who clinically were suspected of syphilis, but who have not returned for further study. In every case in which a positive Wassermann was accompanied by a positive Hinton test, syphilis has been present, and this information alone would make the Hinton test invaluable as a supplementary test for syphilis.

Both reactions were negative in 1825 cases. Eighteen hundred and twelve of these, or 99 per cent, were in patients who had no history or clinical evidence of syphilis. Two cases of central nervous system syphilis under intensive treatment gave negative blood reactions, as did the 11 cases referred to above as cured or latent cases of syphilis. In only 2 cases out of 1825 did the combined negative result occur with active syphilis, another fact which makes the confirmatory evidence of the Hinton test valuable.

There were 13 cases in which the Wassermann reaction was strongly positive and the Hinton test negative. Eight of the 13 were obviously false positive Wassermanns and have had subsequent negative reactions. Of the remaining members of this group, two were young married women without a single sign or symptom of syphilis, whose husbands were nonsyphilitic clinically and serologically, and whose children were not luetic. We are inclined to consider these women as nonluetic, in spite of the fact that each has had three doubtful Wassermanns and two positive Wassermanns, though they are being further studied to determine their true status. One other case gave a history of a questionable sore, but is not available for further study. Another case was one of active meningo-vascular syphilis and was definitely a miss for the Hinton test. A young, pregnant, single girl falls into this group, with three doubtful Wassermanns and one positive Wassermann, but we do not as yet feel certain as to the presence or absence of syphilis in this individual. The outstanding fact in this group is that, of the 13 cases with positive Wassermanns and negative

Hinton tests, eight, and probably ten, cases were mistakes in the Wassermann; whereas the Hinton test was definitely wrong only once and questionably in two instances.

There were 41 cases where the Wassermann was negative and the Hinton positive. Twenty of these were definitely syphilis which the Wassermann missed, and 16 of these 20 were instances of the occasional negative and occasional positive which are often obtained during the course of late treated syphilis, cases apparently not cured of their syphilis. One other case gave a history of a hard chancre with local treatment only, and was missed entirely by the Wassermann. Another instance was that of a 56 year old woman with a saddle nose, saber tibiae, interstitial keratitis, and a history of snuffles and a rash as an infant, an untreated congenital luetic. Another case had a gumma clinically, which responded promptly to antiluetic treatment and, though the Wassermann has never been positive, the Hinton test was positive. An old treated case of tabes dorsalis had also repeatedly negative Wassermanns and positive Hintons. There were seven cases in this group with falsely positive Hinton tests, as proved by autopsy or further clinical and serologic study. The remaining 14 have not been available for further study. It is of interest that 12 of these 14 are over forty-five years of age and suggests that if they could be followed they might be found to be old, "burned out" cases which the Wassermann misses, for many of them have symptoms which would be explained by syphilis.

There were 23 cases with doubtful Wassermann tests and positive Hinton tests and all were cases of proved syphilis, except 4 cases of doubtful diagnosis. On the other hand, there were 36 cases with doubtful Wassermann tests but with negative Hinton tests, and 28 of these were not luetic. Five cases with this latter combination were old treated lueties, one was an untreated asymptomatic syphilitic and one other had tabes dorsalis. The positive or negative Hinton test, then, has been a quite accurate indication of the significance of a doubtful Wassermann.

The Hinton test was doubtful in 2 syphilities with positive Wassermanns and in twenty-four nonlueties and four lueties with negative Wassermanns. Four treated cases of syphilis had both tests return doubtful.

Studying all these groups, we found that the Wassermann was correct in 1995 cases and wrong in 48, while the Hinton test was correct in 2034 cases and wrong in 20. This gives an error of 2.3 per cent for the Wassermann, as compared to 1.3 per cent error for the Hinton test.

Taking the cases with positive Hinton tests as a group, we found that we had 191 cases of known diagnosis and 18 doubtful cases. Of the known cases which had a positive Hinton test, 96.4 per cent had syphilis and 3.6 per cent were nonsyphilitic. We can only speculate upon which way the 18 doubtful cases would swing these percentages, but, from the large number of times that a positive Hinton test in a doubtful case meant a history of syphilis upon further study, it is our impression that these figures would not be greatly altered. On comparison with the Wassermann, we found that only 93 per cent of 160 known cases in which a positive Wassermann occurred were syphilitic and the rest nonsyphilitic.

There were 1874 cases with negative Hinton tests, of which 98.6 per cent were nonluetic, 0.7 per cent were cured or latent cases, and 0.4 per cent were cases of syphilis under active treatment. Only one case was an active, untreated, meningo-vascular syphilitic. Of 1880 cases in which the Wassermann was negative, 98.5 per cent were nonluetic, 0.7 per cent were cured or latent, and 0.7 per cent were cases of syphilis under treatment. There were 3 untreated cases with clinical evidence of syphilis and negative Wassermanns. The two tests differ here only in having more cases of treated late syphilis become Wassermann negative than became Hinton negative.

This brings up the point whether the persistently positive Hinton test in actively treated late syphilis is a real indication of the uncured condition of a

TABLE III

		WASSERMANN	HINTON	INTERVALS BETWEEN TESTS
A.B.	Nonsyphilitic. Acute Prostatitis	Negative	Negative	
		Positive	"	21 days
		Negative	"	3 "
		"	"	4 "
		"	"	7 "
E.H.K.	Nonsyphilitic. Acute Upper Respiratory Infection	Positive	Negative	
		"	"	4 days
		Negative	"	21 "
		"	"	4 "
		"	"	30 "
E.L.	Nonsyphilitic. Catarrhal Jaundice	Positive	Negative	
		Negative	"	30 days
H.R.	Nonsyphilitic. Asthma	Positive	Negative	
		"	"	4 days
		"	"	10 "
		Negative	"	30 "
		"	"	4 "
		"	"	14 "
		"	"	10 "
		"	"	30 "
C.S.	Treated Syphilis	Negative	Positive	
		Doubtful	"	30 days
H.L.	C.N.S. Syphilis	Negative	Positive	
		Doubtful	"	4 days
		"	"	4 "
H.S.	Pregnancy	Doubtful	Negative	
		"	"	4 days
		Positive	"	7 "
		Doubtful	"	3 "
		"	"	30 "
L.L.	Nonsyphilitic	Doubtful	Negative	
		"	"	21 days
		Negative	"	14 "
		"	"	7 "
H.	Syphilitic Glossitis	Doubtful	Positive	
		Positive	"	7 days
H.C.	Syphilis	Positive	Positive	
		Doubtful	"	3 days
		Positive	"	7 "
DeN.	Nonsyphilitic. Asthma	Doubtful	Negative	
		Negative	"	14 days
		Doubtful	"	30 "
		Negative	"	7 "
L.J.	Treated Syphilis	Negative	Positive	
		Doubtful	"	90 "

patient whose Wassermann has become negative, or whether the Hinton test is due to a condition of the blood which occurs in an individual who has or has had syphilis. The fact that the actively treated primary and secondary cases, in which we feel more confident of cure, develop a negative Hinton as readily as a negative Wassermann is against the latter assumption. Time alone can tell us the true significance in the late cases, but the fact that those cases of late syphilis which we have seen develop negative Hinton tests have nearly always been those in whom cure is more likely, and the fact that a few cases in which the Wassermann has been negative and the Hinton positive have later developed signs of activity, make us feel that the Hinton test may prove to be a more accurate estimation than the Wassermann of the cure or arrest of syphilis.

A striking feature of the glycerol-cholesterol test is its consistency. It is well known that the Wassermann test will vary in its degree of positivity from time to time without any treatment at all. Our experience with the Hinton test shows that, with only six exceptions in 2331 tests, the reading once established remained constant. That is, the positive cases remained positive, unless influenced by long, intensive treatment, and the negative cases were persistently negative. Table III gives an idea of the marked difference in consistency of the tests.

In the seven cases of false positive Hinton tests, we found no definite association with acute infection or with any disease, except for the fact that 2 of the 7 cases were instances of cerebral vascular accidents and 2 were cases of diabetes. There was, however, among the erroneously positive and weakly positive Wassermann reactions a large number with acute respiratory infection. Thus, we found in this group three asthmatics during their acute exacerbations, one pneumonia, three acute upper respiratory infections, one lung abscess, one bronchiectasis, and one pulmonary tuberculosis. Only one false positive Wassermann was obtained with jaundice and two with diabetes. The remainder of the false positive and doubtful reactions were in miscellaneous conditions.

SUMMARY

1. There were 2331 consecutive Wassermann and Hinton glycerol-cholesterol precipitation reactions performed on 2120 patients.

2. Both tests agreed in 93.4 per cent of the cases, and in the 6.6 per cent where they disagreed the Hinton test was the one which agreed with the clinical evidence two times out of three.

3. The Wassermann was positive in 72 per cent of the cases of treated and untreated clinical syphilis, while the Hinton was positive in 89.7 per cent of this group.

4. In all cases where both tests were positive, the clinical diagnosis was syphilis; and in 99 per cent of the cases where both were negative, there was no evidence of syphilis.

5. Eleven false positive Wassermann reactions were obtained, largely in cases of acute respiratory tract infection, in which the Hinton test was consistently negative, and only 7 false positive Hinton tests were obtained.

6. The result of the Hinton test was an accurate indication of the significance of the doubtful Wassermann reaction.

7. The Hinton test was very much more consistent in its reading in the individual case than was the Wassermann.

8. In this series, the error in the Wassermann results was 2.3 per cent, as compared to 1.3 per cent in the Hinton test.

CONCLUSIONS

1. In this series of 2120 consecutive cases, the Hinton Glycerol-Cholesterol Precipitation test has agreed with the clinical evidence in more cases than the Wassermann reaction.

2. An adequate serologic examination for syphilis should include the Hinton test.

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A METHOD FOR DETERMINING THE LENGTH OF THE FEMORAL NERVE, AND THE LENGTH OF THE REFLEX ARC INVOLVED IN THE KNEE JERK IN THE DOG*

By E. M. MacEwen, M.D., and W. W. Tuttle, Ph.D., Iowa City, Iowa

THE structure of the arc involved in the "knee-jerk" reflex has been and still is the subject of many conflicting statements. The arguments, pro and con, have for the most part revolved around the conduction time involved in this reflex. Data for measuring this arc are lacking since no exact method was available for determining the length of the femoral nerve. In an attempt to obtain exact data on this, the anterior femoral nerves were measured in nineteen cadavers, from the superficial origin of the motor rootlets to the motor point in the rectus femoris muscle. During this work it was found that the length of this nerve corresponded very closely to the distance from the anterosuperior spine of the ilium to the upper border of the patella. The motor point was also found to lie at a mid-point between these two landmarks, Tuttle and MacEwen (1928).

The results obtained from the use of these data on the human, were so satisfactory, that it was deemed worth while to apply them to some laboratory animal. The dog was selected partly because of its almost universal use, but mainly because the great range in size of this animal afforded ample test for the data.

METHODS

A preliminary experiment was performed on three dogs. The anterior femoral nerve on each side was carefully worked out. Starting where it emerged from the psoas major muscle, the nerve was traced back to its intervertebral

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foramina. The branch to the rectus femoris muscle was isolated and traced to its termination. A laminectomy was done and the roots traced back to their superficial origin. It was found that by carefully cutting away pedicles, the roots could be brought into almost a straight line with the main trunk of the nerve. The branch to the rectus femoris muscle leaves the main trunk shortly after it emerges from the psoas major muscle. This is about a centimeter below the level of the inguinal ligament. The branch then turns laterally at almost a right angle and is accompanied by the vascular supply to the upper part of the muscle. In its course it passes beneath the sartorius to the septum between this muscle and the rectus femoris. In this septum it breaks up into three divisions: superior, middle, and inferior. Each division appears to pass to corresponding one-third of the rectus femoris muscle. They enter the deep surface of the muscle and immediately break up into a number of smaller branches. In all cases the middle division entered the muscle belly of the rectus femoris at a point which varied from a distance half way between the anterosuperior spine of the ilium and the superior margin of the patella to three and one-half cm. proximal to this point. As compared with the human, the motor point in the dog cannot be so accurately found without resorting to stimulation. Because the termination of this middle branch seemed to correspond so closely to the motor point found for the human, it was called the motor point for the dog's rectus femoris muscle.

Under ether anesthesia, the rectus femoris and sartorius muscles were retracted in two dogs. Each division of the femoral nerve to the rectus femoris was isolated and stimulated directly. When the electrodes were placed on the middle division, a maximal contraction of the entire muscle was obtained. The same stimulus applied to the upper and lower divisions resulted in little or no contraction. When the electrode was applied to the outer surface of the muscle, over the points of distribution of each division, similar results were obtained. This substantiated our idea that the entrance of the middle division represented the true motor point.

Five dogs were placed under ether anesthesia, the hair shaved off, the electrode applied to the skin over the rectus femoris muscle, successively from origin to tendon of insertion. In all of these, when the electrode was applied over a point on the skin about a centimeter above the mid-distance between the anterior limit of the crest of the ilium and the upper border of the patella, a powerful contraction of the entire muscle was observed.

In view of the above facts we felt justified in considering the termination of the middle branch as the motor point. In addition it corresponds in relative position to that observed in the human.

In ten dogs the distance from the anterosuperior spine on the crest of the ilium to the upper border of the patella was measured with the leg in extension. The distance to the motor point as above established was also recorded. A complete exposure of the anterior femoral nerve was made on both sides. In order to get the middle branch to the rectus femoris muscle in a straight line with the main trunk and exposed roots, the sartorius and rectus femoris muscles were reflected down from their origins. We were thus able to get the entire nerve.

from the origin of the motor rootlets of the fourth lumbar, to the motor point in the rectus femoris muscle in a straight line.

Table I shows the measurements obtained and a comparison between the length of the femoral nerve and the distance between the anterior limit of the crest of the ilium and the upper border of the patella.

SUMMARY

The dogs used were all adults. In size they varied from large collies to small fox terriers. Inasmuch as the data obtained were so uniform it was deemed unnecessary to extend the series.

TABLE I

A COMPARISON OF THE LENGTH OF THE FEMORAL NERVE WITH THE DISTANCE FROM THE ANTEROSUPERIOR SPINE OF THE ILIUM TO THE SUPERIOR MARGIN OF THE PATELLA

DOG NO.	DISTANCE FROM SUPERFICIAL ORIGIN OF MOTOR ROOTS FROM CORD TO MOTOR POINT		DISTANCE FROM THE ANTEROSUPERIOR SPINE OF THE ILIUM TO THE SUPERIOR MARGIN OF THE PATELLA		DISTANCE FROM THE ANTEROSUPERIOR SPINE TO THE MOTOR POINT		DIFFERENCE BETWEEN ACTUAL NERVE LENGTH AND SURFACE MEASUREMENTS	
	RIGHT LEG	LEFT LEG	RIGHT LEG	LEFT LEG	RIGHT LEG	LEFT LEG	RIGHT LEG	LEFT LEG
	CM.	CM.	CM.	CM.	CM.	CM.	CM.	CM.
1	20	20	20	20	6.5	6.5	0	0
2	22	22	21	21	7	7	1	1
3	18	18	18	18	9	9	0	0
4	24	24	24	24	10	10	0	0
5	21	21	21	21	9	9	0	0
6	13.5	13.5	13.5	13.5	8	8	0	0
7	15	15	15	15	7	7	0	0
8	21	21	21	21	9	9	0	0
9	22.5	22.5	23	23	9	9	0.5	0.5
10	17.5	17.5	18	18	8	8	0.5	0.5
11	16	16	16	16	7	7	0	0
12	18.5	18.5	18.5	18.5	7.5	7.5	0	0

In twelve dogs, the data are comparable to those found for the intact human and warrant similar conclusions as follows:

1. The length of the femoral nerve, from its superficial origin in the cord to its end plate in the rectus femoris muscle is equal to the distance from the anterosuperior spine of the ilium to the superior border of the patella.

2. The motor point of the rectus femoris muscle in the dog is not constant as in the case of the human. In most cases it is proximal to the middle of the muscle and can be located accurately only by the method of stimulation.

3. The length of the reflex arc involved in the knee jerk of the dog is determined by doubling the distance from the anterosuperior spine of the ilium to the superior margin of the patella and adding to this the distance from the point of stimulation to the motor point of the femoral nerve in the rectus femoris muscle.

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THE USE OF BUFFER CITRATE SOLUTION AS A DILUENT AND PRESERVATIVE FOR RED BLOOD CELLS*

By Lester J. Schultz, Ph.D., New York

AFTER observation of the effect of a buffered citrate solution, as used by Dr. Benjamin Jablons[1] upon the red blood corpuscles after intravenous injection, it occurred to me to study the properties of the same solution as a laboratory agent for dilution and preservation of red blood cells. The following is a brief résumé of the studies and observations along this line.

It was observed that whole blood taken from the vein and mixed with this solution in varying proportions from that of equal parts to a concentration of 1 to 200 failed to show any appreciable effect on either the red or white cell components of the blood. After eight weeks exposure at room temperature in this solution, there was no evidence of any disintegration of the red cells or any appreciable change in the white cells. The fact that no chemical change takes place in the protein molecule of either the red or white blood cells, is proved by the following observation.

The addition of a 1 per cent solution of phenol red to the solution containing the blood, even after eight weeks exposure and contact at room temperature, showed no change in the P_H or hydrogen-ion concentration of the solution containing the blood. It was also found that blood cell counts did not vary after a lapse of eight weeks, excepting for the permissible error due to the personal equation. The composition of the solution is as follows:

Sodium Citrate	20 grams
Dibasic Potassium Phosphate	2 grams
Sodium Chloride	3 grams
Doubly distilled water	1000 grams

The P_H should be approximately 7.7. It has also been found that where it is desirable to make counts of the white blood cells, that the addition of methylene blue is somewhat of value. This dye when exposed to room temperature for eight weeks, however, fades somewhat although the blood cells themselves retain their color. Examination of the blood after eight weeks contact with this solution at room temperature, shows the red cells to be unaffected and the white cells completely impregnated with the dye.

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[1]Jablons, Benjamin: Thrombo Angitis Obliterans, Inter. Clinic 111: 193, Sept. 1925.

THE PHENOMENON OF ALCOHOLIC ANTIGEN PRECIPITATION IN LUETIC SERUMS*

By B. S. Levine, Ph.D., Washington, D. C.

Bordet and Gengou[1] published a paper in 1901, the purpose of which was to prove the functional unity of complement. Wassermann, Neisser and Bruck[2] aptly utilized the principle of the unity of complement in the diagnosis of syphilis in inoculated monkeys. Later, in association with Schucht,[3] the same authors extended their new method for the diagnosis of syphilis to human beings. Following the discovery of these authors, Michaelis[4] described a precipitation reaction for the diagnosis of syphilis in which he used serum, a watery extract of syphilitic liver, and normal saline. He considered the reaction specific, believing, as did all serologists of his time, that the syphilitic liver extract contained the specific antigen.

Numerous modifications and new precipitation procedures for the diagnosis of syphilis have since been proposed. Not until recently, however, have any of them proved of sufficient value to be generally adopted. Kahn[5] had demonstrated that by paying heed to certain fundamental conditions, the precipitation of alcoholic antigen with a syphilitic serum could be made to serve the same purpose as does the Wassermann reaction.

The value of the laboratory procedure for the diagnosis of syphilis, whether by the complement-fixation, or by alcoholic antigen precipitation, has been established empirically. The tests, therefore, must still be looked upon as mere adjuncts to the clinical diagnosis of any case under consideration. Nevertheless, sound theoretical reasoning must underlie such laboratory procedures, if the clinician is to depend upon them at all. The gross or basic principles of the Wassermann reaction are at present understood: Antibody, by attaching itself to the alcoholic antigen, sensitizes it, enabling complement to come into play and to complete the formation of the antigen-antibody-complement complex. The phenomenon of antigen precipitation, however, has remained unexplained. It is true, as mentioned above, that Michaelis, as far back as 1907, expressed his belief in the specificity of the reaction, and that Meinecke[6] tried to show that in his third modification of antigen precipitation he was dealing with a phenomenon similar to complement fixation. Yet, neither offered definite experimental proof in support of their beliefs.

Jacob (Else)[7] added complement and the hemolytic system to the completed Sachs-Georgi test, and has concluded from the results obtained that the Wassermann and the Sachs-Georgi reactions are essentially the same.

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However, the author failed to account for the disagreements in the results obtained by such a procedure of experimentation.

Kahn[8] had studied the identity of his antigenic precipitate and of complement fixing substances by a similar procedure. Such a procedure for the study of the two reactions is considered by us inappropriate, and the results inconclusive. There is, in the first place, a certain percentage of disagreement cases which call for an explanation. In addition, the experiments show merely that the two reactions are coincidental in a large percentage of cases, but fail to show analytically that the factors underlying the two reactions are the same.

Jacobstahl[9] showed that the precipitate produced by the precipitation reaction fixes complement, and that the supernatant fluid, if free from sediment, does not fix complement. From this he inferred the identity of luetic precipitins with complement fixing substances.

Keining,[10] on the other hand, having made a comparative study of the results obtained by the Sachs-Georgi precipitation and by the complement-fixation procedures, found that frequently a positive Sachs-Georgi yields a negative superimposed Wassermann, whereas, on the other hand, a negative Sachs-Georgi may result in a positive Wassermann. He, therefore, came to the conclusion that the antigen precipitation and complement-fixation reactions depend upon different serum constituents.

On the basis of discrepancies in the results obtained by the Kahn precipitation and by the complement fixation procedures, Kilduffe[11] also suggests a difference either in the mechanism or in the substances involved in the two diagnostic procedures.

Not until the true nature of the antigen precipitation by luetic serum is well established, will it be possible to intelligently discuss the results of the precipitation and of the complement fixation on a comparative basis. With a view to obtaining first hand information on the subject under consideration, I have made several series of elaborate experiments described herein.

A number of serums have been tested by the following three methods: (1) U. S. Veterans' Bureau[12]; (2) Kolmer[13]; (3) Kahn.[14] The racks containing the Kahn tests of the serums which proved positive by the three tests mentioned, were properly marked and placed in the ice box overnight. They were subjected to prolonged centrifugation in order to throw down the precipitates. The supernatant diluted serums were poured off into another set of tubes marked to correspond with the original. The two sets of the tubes were placed into separate racks marked "P," for precipitate, and "S" for supernatant fluid. To each tube of the "P" set, one mil of 0.85 per cent saline was added, and the precipitates resuspended by agitation. The contents of the tubes of both sets were tested by the complement-fixation procedure. It was found that the complement remained free in the supernatant fluids, but was completely fixed by the precipitates. This was in agreement with the results of Jacobstahl.

From a consideration of the known sero-immunologic principles, complement fixation and resulting inhibition of hemolysis occur only in the union

of antigen with antibody. And since only one kind of antibody is capable of uniting with the same antigen, the experiment affords reason to suspect that the precipitation of the antigen was caused by the antibody present in the serologically positive serums.

In the above experiments some of the supernatant fluids containing the serum remained in association with the sedimented precipitates. To eliminate the influence which might have been exerted by the traces of serum upon the fixation of the complement, a set of positive serums had been treated as described above. The sedimented antigenic precipitates, then, had been washed with saline and centrifuged. This step had to be carried out with extreme caution. Washing the sediment with saline may cause it to become resuspended in the wash-saline in an inseparable condition. The tubes containing the sediment, the wash solution, and the original supernatant fluid were tested by the complement-fixation procedure. Complete fixation of the complement was manifest in the tubes containing the washed precipitate, and complete hemolysis in the tubes containing the original supernatant fluid and wash-saline. The fixation of complement in the tubes containing the sediment in the first experiment, therefore, was not due to the presence in those tubes of traces of the positive serum. It was caused by the absorption of the complement by the sedimented antigen-antibody complex.

The results of the above experiment demonstrate that alcoholic antigen is precipitated by, so-called, positive serums as a consequence of the formation of an antigen-antibody complex, which is capable of absorbing complement. Since, as stated above, the antigen used in the complement-fixation tests was prepared practically the same way as the antigen used in connection with the precipitation tests, it follows that the antibody involved in the antigen precipitation reaction is identical with the antibody concerned in the Wassermann reaction. It appears valid, therefore, to conclude that antigen precipitation demonstrates the same immunologic state of the serum tested as does complement fixation.

Similar results by the use of a similar procedure were obtained by Stühmer and Merzweiler[15] in connection with a study made by the authors on the Sachs-Georgi and the Wassermann reactions. They concluded, as a result of their experiments, that contrary to the opinion of Keining,[10] "dislipoidization" is not the factor which inactivates the "reagin," but that the reagin is removed from the serum-extract mixture by the antigenic precipitate.

In a critical discussion on the validity of testing the antigenic precipitates by procedures similar to those used by us and other investigators and of the conclusions drawn from the results obtained, Kafka[16] concludes that such experiments do not demonstrate a fixation of complement in the same sense as it is understood to occur in the Wassermann reaction, but that a purely physical destruction of the complement, incident to its adsorption by the suspended precipitate, takes place. Rothman[17] had used barium sulphate and copper hydroxide in saline solution, and by appropriate precipitation and dilution suspended them so that agglutinoscopically they resembled a straight Sachs-Georgi reaction. He found that such precipitates fixed complement,

while the filtrates did not fix complement. He concluded, therefore, that the hemolytic inhibition caused by the precipitated serum-extract mixtures indicated no specific complement fixation. Like Kafka, he considered that such inhibition was due to the physical destruction of the complement caused by its adsorption by the macroscopic precipitate.

The error of Rothman's conclusion evidently arises from the fact that he deals in his experiments with strictly nonspecific precipitates. However, to prove his and Kafka's errors, experimentation was required. In connection with the study of the principles controlling alcoholic antigen precipitation, I have found the following: When cholesterinized alcoholic antigen is precipitated with an equal volume of 0.85 per cent salt solution, placed in proper quantities into serologic tubes, followed by 0.15 c.c. of serum, shaken for three to five minutes, as prescribed by Kahn, and then 1.0 c.c. of distilled water instead of saline is again added, the tubes shaken, and allowed to rest overnight, the alcoholic cholesterinized antigen is precipitated from the negative as well as from the positive serums. By centrifugation the removal of the supernatant fluids is accomplished with ease and completeness.

Having established the constancy of the phenomenon just described, the following sets of experiments were carried out: Negative and positive serums were subjected to reaction with antigen as per the Kahn procedure. The antigen was, then, precipitated out with distilled water as described above. The tubes were allowed to stand overnight in the ice box. The following day, they were thoroughly centrifuged, and the supernatant fluid removed from the sediments. To the tubes containing the supernatant fluids, 8.5 per cent saline was added to the extent of 0.1 c.c. per 1.0 c.c. of distilled water, to restore the original tonicity. The tubes containing the precipitates and the supernatant fluids, then, had been tested for complement fixation. No complement was absorbed by any of the supernatant fluids nor by the precipitates obtained from the negative serums.

In another set, a number of negative serums were treated with cholesterinized alcoholic antigen exactly as described above. After having separated the precipitates from the supernatant fluids, one-half of the precipitates were resuspended in 0.85 per cent saline, while to the precipitates of the other half appropriate quantities of a number of positive serums were added. The tubes were well shaken for three to five minutes, and the cholesterinized antigen reprecipitated from the positive serums with distilled water. The sediments thus obtained were again separated from the supernatant fluids. After proper adjustment for tonicity, all the supernatant fluids and the precipitates had been tested for complement fixability. The complement remained free in all the tubes containing the supernatant fluids as well as in the tubes containing the precipitates obtained from the negative serums which had not been subjected to any further treatment. The complement was completely fixed by the precipitates originally obtained from the negative serums and later treated with positive serums.

The results of these sets of experiments show conclusively that the mere physical condition of the antigenic precipitate does not sensitize it to the

absorption of complement. Only in the union with syphilitic antibody does the precipitated antigen fix complement.

To further substantiate this conclusion, the following experiment was carried out. A 0.5 per cent alcoholic solution of pure cholesterol was prepared. Two mil. of this solution were mixed with an equal volume of 0.85 per cent saline. A flocculent precipitate resulted. Five hundredths c.c. of the cholesterol-saline mixture was placed into each of a series of serologic tubes. Fifteen hundredths c.c. of each of a number of positive and of negative serums, then, had been placed into the tubes, and the racks well shaken for three to five minutes. One mil. of distilled water, then, was added to each tube, the racks again shaken and left stand in the ice box overnight. The following day the tubes were centrifuged, the supernatant fluids removed from the precipitates, and after proper adjustment for tonicity, the supernatant fluids and the precipitates had been tested for complement fixation.

The complement remained free in the tubes containing the supernatant fluids from the negative serums, and in the tubes containing the precipitates from both, the negative as well as the positive serums. The complement was completely fixed in the tubes containing the supernatant fluids obtained from the positive serums. The above experiments vividly show that nonspecific flocculent colloidal precipitates obtained from either positive or negative serums do not fix complement, because they do not remove with them the luetic antibody from the positive serums. It is, therefore, valid to conclude that the precipitates yielded by the Kahn positive serums, possessing complement fixing properties, represent the antigen-antibody complex. Furthermore, since the removal of the precipitates renders the supernatant fluids Wassermann negative, it is likewise valid to infer that the same antibody that is responsible for the fixation of the complement in the Wassermann reaction, is responsible for the precipitation of antigen by the Kahn and other precipitation procedures.

CONCLUSIONS

1. The phenomenon of antigen precipitation in the Kahn and in similar reactions is a manifestation of the formation of an antigen-antibody complex.

2. The same antibody that is concerned in the complement-fixation test is responsible for the phenomenon of antigen precipitation in luetic serums.

3. Antigen precipitation in syphilis, therefore, is a direct manifestation of the first step of the Wassermann reaction; namely, of the union of alcoholic antigen with the luetic antibody.

4. The difference in the results obtained by the various complement fixation and antigen precipitation procedures, therefore, is not due to any difference in the principles upon which the reactions depend or of the primary substances involved. It is the difference in the concentration of the complex reagents used, a factor which profoundly affects the balance of the colloidal system under study, that causes the discrepancies.

This phase of luetic serology will form the subject matter of a following paper.

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AN ALKALI RESISTANT STOPCOCK*

By F. C. Koch, Ph.D., Chicago

STOPCOCKS made of black, hard rubber have been used in glass burettes containing 10 per cent and 40 per cent sodium hydroxide respectively and have been found satisfactory in every respect. There never has been any indication of the stopcocks sticking or any suggestion of developing leaks. Such stopcocks are relatively easily prepared on the usual lathe equipment found in laboratory shops.

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A NEW SET OF POTASSIUM DICHROMATE STANDARDS FOR DETERMINATION OF THE ICTERUS INDEX*

By Charles C. Farahaugh and Grace Medes, Minneapolis, Minn.

THERE is a growing conviction among those concerned with the estimation of quantitative variations in serum bilirubin, that the determination of the icterus index offers a more satisfactory method than the more laborious and less accurate quantitative modification of the van den Bergh.[1]

The determination of the icterus index, as originally worked out by Meulengracht,[2] was accomplished by dilution of the plasma until it matched a given standard consisting of a 1 to 10,000 aqueous solution of potassium bichromate.

A number of modifications of this method have been made by other workers. The most widely used modification seems to be that in which the color comparison is made in an ordinary colorimeter. The same standard (1:10,000 solution of potassium bichromate) is used. The serum is diluted with physiologic salt solution until it nearly matches the standard, the colorimetric reading is made, and the factor, $\frac{\text{standard reading}}{\text{unknown reading}}$, is multiplied by the dilution. This method can be employed only in laboratories where colorimeters are available, and it has the disadvantage that at least 5 c.c. of serum are needed to make a reading.

A more satisfactory method is that proposed by Murphy[3] in which a series of test tubes are used filled with bichromate solutions, the concentrations of which are as follows: 1, 2, 5, 10, 15, 20, 25, 50, 75 and 100 parts of potassium bichromate in 10,000 parts of water, corresponding to indices of 1, 2, 5 - - - 100. A quantity of serum is placed in a similar glass tube and by comparison its index value is determined without dilution.

There are two weaknesses in this method. The first is that it attempts to cover the whole range of index values in a single series and leaves too large gaps between the tubes. For instance, it is generally agreed that the serum of normal persons gives an index value of 4 to 6, and that when skin icterus becomes just visible, the reading is 15. The range between these two values, 5 to 15, has been designated as that of "latent jaundice." Obviously it is in this range that icterus index readings are most valuable. The series of standards as arranged by Murphy gives only a single value within this range, and is therefore not adapted for quantitative estimations, the first standard above the normal being 100 per cent greater than normal.

The second weakness of the Murphy method lies in the fact that in the standards of highest concentration the yellow color becomes so intense that variations are difficult to observe and the more nearly the color approaches saturation, the greater the error. In a series of investigations carried out in

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our laboratory we found that the point at which this error becomes appreciable is just above an index of 10 and that the error increases rapidly as an index of 100 is approached.

To obviate these two drawbacks of the Murphy method, we have devised a set of standards covering a range of 1 to 10. Tube 1, as in both the Meulengracht and Murphy methods contains a solution of 1 part potassium bichromate in 10,000 parts of water slightly acidified with a few drops of H_2SO_4. The following series is employed:

ICTERUS INDEX	$(K_2Cr_2O_7):(H_2O)$
1	1:10,000
1.5	1.5:10,000
2	2:10,000
2.5	2.5:10,000
3	3:10,000
3.5	3.5:10,000
4	4:10,000
4.5	4.5:10,000
5	5:10,000
6	6:10,000
8	8:10,000
10	10:10,000

These standards are permanently sealed in clear glass tubes of about the diameter of Wassermann tubes, and a similar tube is used to contain the serum to be compared. In case the serum is of a deeper color than corresponds to an icterus index of 10, one or more dilutions must be made until the reading of the diluted serum lies somewhere within the range of the standards. Thus with an index of 20 only one dilution would be necessary, and with an index of 40 or 80, 2 or 3 would suffice instead of the 5 or 6 which would be required by the Meulengracht method. Dilution is made with a physiologic salt solution in order to prevent precipitation of the serum globulins. The intervals between the tubes are sufficient so that a definite difference between the colors of any two tubes may be observed and yet variations even in normal sera may be ascertained.

In case there is any doubt as to the substance in serum responsible for the darkened color, a qualitative test for bilirubin should be made. We have found the Gilbert[4] modification of the Gmelin test most satisfactory.

SUMMARY

A modification of the Meulengracht and Murphy methods for the determination of icterus index is described. It consists of comparison with a set of standard solutions of potassium bichromate corresponding to indices ranging from 1 to 10.

These standards cover the range of normal variations, a subnormal zone from 4 to 1, and a slightly elevated zone from 6 to 10.

Serum pigmented more deeply than corresponds to an index of 10 must be diluted until it lies within the range of the set of standards.

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A NOTE ON THE INTRAPERITONEAL METHOD OF INDUCING ANESTHESIA WITH BARBITAL Na IN THE DOG*

By C. A. Johnson,† Chicago, Ill.

In 1922 Parsons and Tatum[1] described the oral use of barbital as an anesthetic agent for dogs. Since that time Pearcy and Weaver[2] indicated and discussed the various methods of inducing anesthesia with this drug and some of its derivatives.

For the past two years we have used barbital Na as an anesthetic in acute dog experiments and have found it very satisfactory when given by the intraperitoneal route in similar doses suggested by Pearcy and Weaver (250 mg. per K.). In most cases anesthesia occurred in from fifteen to thirty minutes until suddenly we had a great number of failures, i.e., with the same doses per K body weight, anesthesia would not occur for several hours, if at all.

At first we attributed these failures to the drug being of poor quality and this led us to obtain another preparation from a different drug house. Using this, we still continued to have poor results. It then occurred to us that the site of injection had been changed. In the successful cases the upper abdominal quadrants had been used for injection while in the unsuccessful ones we had used the lower quadrants. Further observations as indicated in Table I which contains the more or less quantitative data in support of the contention.

Table I

WT. OF ANIMAL	AMOUNT OF DRUG PER K.	SITE OF INJECTION	ANESTHESIA IN:
15 K.	250 mg.	Just below the Xiphoid	26 minutes
11 K.	250 mg.	Just below the Xiphoid	24 minutes
15 K.	250 mg.	Just below the Xiphoid	16 minutes
15 K.	250 mg.	Right lower quadrant	2 Hours and 16 min.
11 K.	250 mg.	Left lower quadrant	1 Hour and 42 min.

It has been stated and observed clinically that absorption from the upper abdominal cavity is more rapid. We can interpret these data on the basis of a more rapid absorption by the visceral and parietal peritoneum of the upper abdominal cavity.

We have written this note, hoping that the suggestion for the administration given above will save time and prevent disappointment of those investigators who wish to use the intraperitoneal method of inducing a satisfactory anesthesia with barbital Na.

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RAPID AND ACCURATE BLOOD CHEMISTRY DETERMINATIONS*

By W. G. Gamble, Jr., M.D., Charleston, S. C.

BLOOD chemistry determinations are now part of the routine laboratory procedures. This has increased the work of the laboratory personnel without materially increasing its numerical strength. Hospitals and physicians request emergency blood chemistry determinations, such as, blood sugars, and

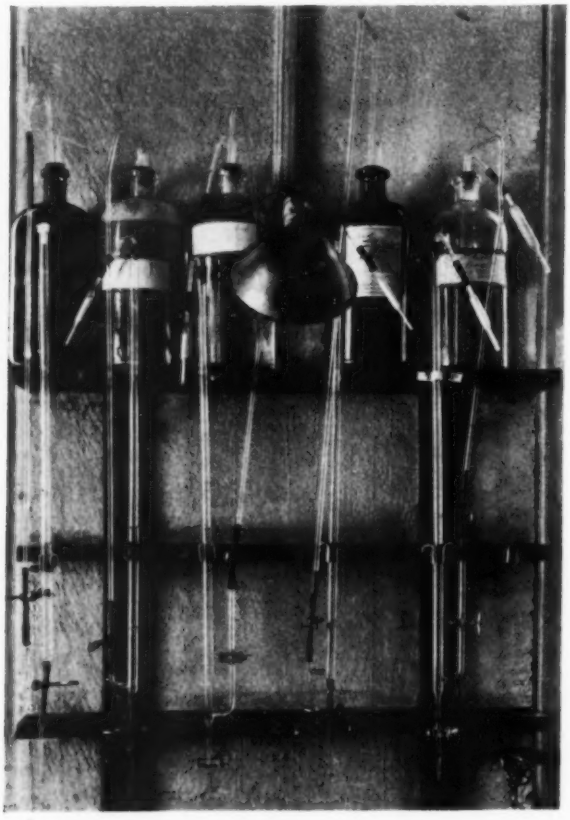

Fig. 1.

they desire reports in the shortest possible time. These determinations are at best time consuming, and especially is this so when a number of specimens are submitted.

Fig. 1 represents a device which may not be new, but as far as I know it has not been pictured. It is especially adaptable to the Folin-Wu methods and decreases the time element on single, as well as on numerous specimens.

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Each apparatus consists of a two gallon jug connected by a siphon glass tubing and suitable pinchcocks, to an automatic refillable burette of 50 c.c. capacity graduated accurately to 0.1 c.c. All air vents are protected by trap tubes containing soda lime. The other details are self-evident. For blood-sugar determinations a battery of five is used. First, Container of distilled water. Second, 10 per cent sodium tungstate. Third, 2/3 N sulphuric acid. Fourth, Folin copper tartrate solution. Fifth, Folin molybdate acid solution. Others for Nessler's solution etc., may be added so this method may be used in other chemical determinations, for in our hands, it has proved readily adaptable.

Slow pipetting is avoided and rapid precipitation accomplished. We find it possible to complete blood-sugar determinations in twenty-five minutes.

This method has been checked repeatedly and the results are practically the same.

A SIMPLE METHOD FOR SLOW INTRAVENOUS INJECTIONS*

By Ralph G. Smith, M.D.,† and Walter E. Gower, M.A., Chicago, Ill.

IN LABORATORY work it is sometimes desired to make continuous intravenous injections at a very slow rate. For this purpose the apparatus described below has been adopted with satisfactory results. By using the passage of mercury through a stopcock as the controlling factor it is possible to attain a much slower rate than by the direct injection of an aqueous solution from a burette. By the method to be described the injection may be slowed to a rate of 1 c.c. in twenty minutes.

Fig. 1 shows the essential features of the apparatus. It consists of a burette with a stopcock and two similar bottles, A and B, of such a size that one will contain the volume of the fluid to be injected. They are assembled with bored rubber stoppers and tubing as represented in the diagram. In preparation for use A is filled with the solution to be injected, B with paraffin oil, and the burette with mercury. The mercury is allowed to drop into B thus displacing the oil through X into A. Air bubbles are driven from B in front of the oil and are forced from the system through Y which remains open while Z is closed. On closing Y and opening Z, which is connected with a hypodermic needle, the injection fluid is forced from the latter at the desired rate which is controlled by the burette stopcock. The volume of fluid injected is determined by the fall of mercury in the burette. As the injection proceeds the rate will be somewhat slowed by such a fall, due to a decrease in pressure. This, of course, may be rectified by readjusting the stopcock or by refilling the burette at frequent intervals. However, if it is essential that the rate remains constant throughout, mercury may be allowed to drop from a second burette into the first burette at such a rate that the level in the latter remains practically constant. The volume of fluid injected is then determined by reading both burettes.

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If desired, an attachment may be added for refilling the apparatus without dismantling it. A mercury leveling bulb is connected with a Y-tube, one arm of which passes through the rubber stopper to the bottom of B. By lowering the leveling bulb the mercury is siphoned from B and at the same time A is refilled with the injection fluid by drawing it in through Z. The second arm of the Y-tube from the mercury leveling bulb may be fused to the burette just above the stopcock and used for refilling the burette with mercury. During the injection both arms of the Y-tube are of course closed. With such an

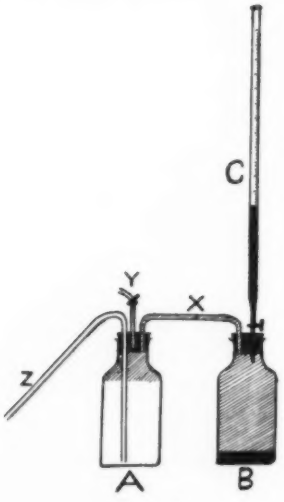

Fig. 1.

attachment the system resembles the Trendelenburg apparatus for recording blood pressure.

The use of bottle B and of paraffin oil is merely for the purpose of preventing the injection fluid from coming in contact with mercury. If such is considered unimportant the mercury may be allowed to drop directly into A with tubing Z passing only to the lower surface of the stopper.

The advantages of the system are its simplicity, the slow constant rate of injection possible, and the ease with which the rate may be varied.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LABORATORY TECHNIC

GASTRIC ULCER: The Acid Test in Gastric and Duodenal Ulcer, Palmer, W. L. J. A. M. A. 88: 1778, 1927.

The test that has been evolved is conducted as follows: If it is to be made at a time when the stomach is thought to contain food, this should be removed through an Ewald tube. A Rehfuss tube is then swallowed by the patient, and suction is made on the syringe in order to make sure that the stomach is completely empty. Any ulcer distress that may have been present is usually gone by this time. If distress continues, the stomach may be washed through the Rehfuss tube, plain water or alkaline solutions being used. As a rule, emptying the stomach is sufficient in itself to relieve the pain, although in cases with a rapid and high grade gastric secretion, repeated emptyings may be necessary. Distress that cannot be relieved by emptying the stomach or by continued alkalization over a period of an hour is almost never ulcer distress. When the patient is free from distress and the stomach empty, one proceeds to the test and introduces through the tube 200 c.c. of 0.5 per cent hydrochloric acid. (This may be made up approximately by using 15 c.c. of concentrated hydrochloric acid, i.e., from 35.4 to 37.5 per cent, to 1,100 c.c. of tap water.) If no pain develops, another 200 c.c. is given thirty minutes later, followed, if necessary, by a third 200 c.c. at the end of the hour, making a total of 600 c.c. The period of observation is usually an hour and a half, and if the patient's typical distress is not reproduced during this time, the test is considered negative. If distress does arise, it is relieved as soon as is deemed desirable by emptying the stomach.

The patient should be told merely that he is to keep the observer informed of the appearance or disappearance of his typical distress. He should not be told that the acid is likely to produce pain.

The "acid test" under the proper conditions, is usually positive in cases of gastric and duodenal ulcer, and in certain cases of gastric carcinoma.

A negative test does not necessarily exclude the presence of these lesions.

The test has been negative in all cases studied so far which did not involve an ulcerative lesion of the stomach or adjacent duodenum (or jejunum in case of gastrojejunal ulcers).

The test is not infallible, but when properly done it possesses a high degree of reliability.

During the course of treatment the test is of value in that when persistently positive it betokens, in my experience, a persistently open ulcer, whereas an invariably negative test is compatible with, but not necessarily indicative of, healing.

AMEBIASIS: The Value of Cultural Methods in Surveys for Parasitic Amebae of Man, Craig, C. F., and St. John, J. H. Am. J. Trop. Med. 7: No. 1, 39, 1927.

This survey, in which 71 individuals were examined, demonstrates that cultural methods are superior, for diagnostic purposes, to either the sedimentation method or the direct examination of the feces, when only one microscopic preparation is examined.

The survey demonstrates that the Locke serum medium gave a larger percentage of positive results than did any of the other methods employed, and that neither coagulated egg nor a solid substratum of any kind is necessary for success in surveys for the intestinal amebae.

The successful results with a medium composed of seven parts of normal salt solution and one part of inactivated human blood serum demonstrate that none of the

chemicals in either Locke's or Ringer's solution, with the exception of sodium chloride, were essential in the media used in the survey and that the continued cultivation of Entameba histolytica over an indefinite period of time is possible in this simple medium, as shown previously by Craig.

The formula for the medium follows:

The Locke solution in this medium has the following formula:

Sodium chloride	9.00 gm.
Calcium chloride	0.24 gm.
Potassium chloride	0.42 gm.
Sodium bicarbonate	0.20 gm.
Dextrose	2.5 gm.
Distilled water	1000 c.c.

The method of preparing this medium follows:

The Locke solution is filtered and autoclaved at 15 pounds pressure for fifteen minutes and then allowed to cool. Four whole eggs are emulsified in 50 c.c. of the solution. The mixture is tubed and coagulated in slants, and to each slant is added sufficient inactivated horse serum diluted with 8 parts of the Locke solution to cover the whole or part of the slant. The tubes are then incubated at 37° C. for several days to test for sterility. If sterile, they are kept in the incubator at 37° C. until used.

LOCKE-SERUM MEDIUM

The Locke solution used in this medium is the same as that used in the preparation of Locke-Egg Serum medium, with the exception that 1 gram of dextrose is used instead of 2.5 gm. The solution is filtered and autoclaved as described above. There is then added one part of human blood serum inactivated by heating in a water-bath at 50° C. for one-half hour, to each 7 parts of the Locke solution. After adding the blood serum, the mixture is thoroughly shaken and filtered through a Mandler or Berkefeld filter. It is sometimes necessary to filter the mixture through two or more candles in order to obtain a perfectly clear filtrate. After filtration, the medium is tubed, placing from 5 to 10 c.c. in each tube, and incubated at 37° C. for several days to determine sterility. If found sterile, the tubes are kept in an incubator at 37° C. until used. The reaction of the medium does not need adjustment.

TYPHOID FEVER: Otani Reaction: Study of Typhoid Fever, Hilario, S., and Ira, F. Rev. Fi. med. y. farm. 16: 244, November, 1925.

Otani's reaction is based upon phagocytosis and characterized by intensification of opsonic capacity under the stimulus of bacterial antigens.

According to Otani the normal phagocytic index is not over 10 per cent. He finds, in typhoid fever, tuberculosis, and dysentery, an increased index strictly specific for these organisms but not for related organisms.

He claims that it has a great advantage over Widal's reaction because it becomes manifest much sooner than the latter and is more sensitive and yet there are no false reactions. In typhoid fever Otani's reaction appears on the second or third day of the disease, that is, within the first week, being in this respect as rapid and efficient as hemo-culture. The technic is simple and consists in placing 4 or 5 drops of blood in a test tube which contains a drop of sodium citrate solution and shaking the tube until it is well mixed. A small quantity of the liquid is taken with a fine pipette and an equal amount of a fine emulsion of the bacillus of the disease to be studied added to it. These two solutions are mixed in a capillary tube by the ordinary method and the tip of the tube closed, after which it is put in an incubator at 37° or in a water-bath at 38°. After ten minutes it is removed and the tip of the capillary tube broken off and a drop of its contents placed on a slide, making a smear. When the smear is dry it is stained with Wright's, Otani's or Giemsa's stain. The leucocytes take the basic color and the microorganisms the violet color. It is examined with the immersion lens, the leucocytes which contain bacteria being put down in one column and the leucocytes free of bacteria in another, at least 100 leucocytes being counted, the exactness of the results

being proportional to the number of leucocytes counted. Any average larger than ten is considered positive and any less than or equal to ten negative. The blood should be fresh as the leucocytes lose vitality if they are kept for some hours, even if it is kept at body temperature. The emulsion should be fine and made of a culture not more than twelve to twenty-four hours old. The saline solution must be isotonic. The sodium citrate solution is a 5 per cent solution in physiologic salt solution. All the apparatus used should be free of fat and alkali, as these substances slow phagocytosis.

BILE ACID IN BLOOD: A New Colorimetric Method for the Quantitative Determination of Bile Acid Salts in the Blood, Szilard, P. Biochem. Ztschr. 173: 440, June 25, 1926.

REAGENTS

1. 1 per cent aqueous solution ferric chloride 1000 c.c.
   Conc. HCl (sp. grav. 1:13) 0.5 c.c.
   This solution is stable if kept in dark bottles.
2. Sulphosalicylic acid c.p. 20 gm.
   Distilled water 100 c.c.
3. Standard Solution:

Place 0.44 gm. of c.p. ferric ammonium sulphate in a liter graduated flask, add 950 c.c. of distilled water, 1 c.c. of concentrated HCl (sp. grav. 1:13) and make up to 1000 c.c.

Filter. Transfer 100 c.c. to a graduated liter flask, add 0.9 c.c. of HCl (sp. grav. 1:13) and make up to 1000 c.c. This solution is stable for two to three months.

To 5 c.c. of this last solution in a test tube add 0.2 c.c. of the 20 per cent sulphosalicylic acid solution. Allow to stand ten minutes and place in the colorimeter. This amount gives a rose-red color equivalent to that given by 0.0625 mg. of sodium glycocholate.

METHOD

Secure blood by venipuncture and oxalate. Centrifuge and add 5 c.c. of the plasma drop by drop to 250 c.c. of absolute alcohol with constant agitation of the flask. Boil, while shaking, over a water-bath for three minutes and filter. Rinse the beaker or flask with 3 portions of hot absolute or methyl alcohol and add the rinsings to the filter.

Distil off the alcohol until the volume is reduced to 10 to 15 c.c. Cool and add 300 c.c. of ether slowly, with constant agitation. Allow to stand until turbidity clears. Decant the alcohol-ether and wash the precipitate with 3 or 4 changes of ether. Decant the ether and evaporate to dryness on a water-bath.

Dissolve the precipitate in 5 c.c. of cold distilled water. Centrifuge, if necessary to clear, and add 0.3 to 0.5 c.c. of ferric chloride solution. Place in the incubator until the fluid above the precipitate is clear and transparent.

Centrifuge and wash the precipitate twice with distilled water. After the last centrifuging add 5 c.c. of water, shake, and pour into a large test tube. Wash the centrifuge tube with 5 c.c. of alcohol and add to the large tube. Mix and add 5 c.c. of chloroform. Mix and add 0.2 c.c. of 20 per cent sulphosalicylic acid. Mix and add 5 c.c. of water containing 0.5 c.c. HCl (sp. grav. 1:13) per 1000 c.c. Shake and set aside until the water (colored red) and the chloroform have separated.

Evaporate the chloroform by pouring warm (50°) water over the tube.

Compare the watery red solution with the standard in a colorimeter.

Calculation: $x =$ unknown
$z =$ standard content of bile salt (0.0625 mg.)
$H =$ height of standard (25 mm.)
$K =$ unknown solution

$$x = \frac{H}{K}$$

Carrying out the multiplication, 1.5625 must be divided by the number of millimeters read corresponding to the unknown quantity.

With this technic bile acid salts can be demonstrated in small amounts in normal blood.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*The Young Man and Medicine**

THE successful business man has recognized for years that the average college student upon graduating knows not what he wishes to do and is equipped for no particular line of life work. University authorities are gradually coming to realize these same facts. One of the most progressive signs of the times in university education is the radical step which has just been taken by the President of the University of Michigan in the formation of a two year academic course at the end of which time the student must have made a decision as to preferential studies.

And now we have orientation courses in which the student learns briefly of the general character of the various professions, and we have series of lectures by outstanding men describing the fields covered in the work in which they find their special interests.

The next logical development would be the appearance of a series of articles or books which will be available for the young man and in which he may read of the work which is done in each profession and be guided in reaching his own decision.

The vocational series edited by Dr. E. Hershey Sneath of Yale consists of a series of twelve volumes. These cover the fields of law, the ministry, teaching, medicine, journalism, banking, business, mechanical, electrical and civil engineering, farming, and government service.

No two men would write a volume on The Young Man and Medicine from exactly the same point of view. Dr. Barker as he usually does has made his volume tremendously interesting and thoroughly complete although perhaps a little too technical to inspire the enthusiasm of a young college student.

We should have liked to have seen a little more of the history of medicine and quite a lot more of stimulating biographic sketches of the great men of the profession. These personal differences of opinion as to how such a book should be written, aside however; it is a volume which should be carefully read by every young man who is considering the profession of medicine as a life career.

Percival's Medical Ethics†

THOMAS PERCIVAL lived between 1740 and 1804. We owe to him the foundation of our present system of medical ethics. Professor Leake has reprinted and reviewed critically Percival's principles and compared them with the original oaths of Hippocrates and the principles of medical ethics as they have been propounded by the American Medical Association. It is well that this has been done by a man who is neither in practice nor a physician but who is well acquainted with the customs of the profession. He points out the

*The Young Man and Medicine. By Lewellys F. Barker, M.D., LL.D., Professor Emeritus of Medicine Johns Hopkins University. (Vocational Series edited by E. Hershey Sneath, Ph.D., LL.D., Yale University.) Cloth. Pp. 202. The Macmillan Company, New York, 1928.

†Percival's Medical Ethics. Edited by Chauncey D. Leake, Associate Professor of Pharmacology, University of Wisconsin, and Lecturer in Pharmacology, University of California Cloth. Pp. 291. The Williams & Wilkins Company, Baltimore, Md., 1927.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

weak points in our system, probably the greatest criticism of which is that the principles are designed more for the protection of the physician than of the patient.

True, there are instances in which the matter of so-called ethics is much over done, sometimes unfortunately not to the benefit of the patient, but after reading Professor Leake's criticisms and re-reading the present-day principles of medical ethics the reviewer comes to the conclusion that this would be a sad profession indeed and that the patient would on the whole be far worse off did we not possess a concrete system very much in its present form. Possibly we have reached the point where it would be well for the American Medical Association to review its principles altering them where desirable in the interests of the patient but we venture to predict that there would be relatively few changes. The trouble is not with the principles as much as with their practice. The conscientious physician applies the spirit of the principles as he should. The ignorant physician is helped to remain along the straight path by their existence. The unscrupulous physician would not be influenced by even the most perfect system of medical ethics. The trouble is that the purist who has had no experience in the practice of medicine is not aware of the gross ignorance of even the intelligent laity on subjects medical. Even to some of our most intelligent citizens the chiropractor and the osteopath are as reliable authoritative sources of knowledge as are the professors in our best medical schools. The well meaning talkative doctor is misquoted perhaps oftener than any other type of man. This is especially true when two or more physicians are attending the same patient. Experience has shown that if the patient or relatives are fully cognizant of minor disagreements among the physicians the mental reaction works to the disadvantage of the patient. And when there are real disagreements or when it is a matter of therapeutic inefficiency one will find in the existing principles of medical ethics ample provision for the protection both of the patient and the physician.

*Modern Aspects of the Diagnosis, Classification and Treatment of Tuberculosis**

IT IS refreshing to find a writer on tuberculosis who speaks of healing as well as quiescence and arrest. Dr. Myers presents an excellent review of the present situation and outlook in tuberculosis from the clinical viewpoint. After a brief concise history of tuberculosis and another chapter on the history of treatment of tuberculosis we find a description of the organism, the method of entrance into the body, diagnosis with tuberculin and a history of the clinical course of the disease. The chapters on diagnosis assume a preliminary acquaintance with the methods of general physical diagnosis and pass at once to the finer details of the recognition of the disease. Fully one-half of the volume is devoted to treatment.

We should say that for the general man, not the specialist in tuberculosis, the volume under review possesses two outstanding values, first, for his assistance in the early recognition and diagnosis of tuberculous infection and second for the application of the proper principles of treatment after the patient has returned from his preliminary course of treatment at the sanatorium, to remain under the supervision of his own doctor.

Pernicious Anemia†

MILESTONES in our knowledge and understanding of specific diseases are marked by monographic treatises, summaries of preexisting knowledge and presentations of recent developments.

The volume on Pernicious Anemia by Beaumont S. Cornell represents such a milestone, for while we still know nothing of the etiology of the disease therapeutic progress has taken such a forward step and interest in the disease has been so enhanced, that a monographic review is most timely.

*Modern Aspects of the Diagnosis, Classification and Treatment of Tuberculosis. By J. Arthur Myers, Associate Professor of Preventive Medicine, Medical and Graduate Schools, University of Minnesota. With an Introduction by David A. Stewart, Associate Professor of Medicine, Manitoba University. Cloth. Illustrated. Pp. 271. The Williams & Wilkins Company, Baltimore, Md., 1927.

†Pernicious Anemia. By Beaumont S. Cornell, M.B. (Tor.), Fellow in Duke University. Cloth. 311 pages. Duke University Press, Durham, N. C., 1927.

The author points out how much of our supposed understanding of the disease is pure speculation. At the same time the accumulation of actual facts most of which have not been successfully interarticulated is quite enormous.

The volume is based on a review of 827 contributions to our understanding of the disease and in spite of the enormousness of the literature the author appears not to become lost in a maze of observations but presents a clear discussion of pernicious anemia. This is the first monograph on the disease which has been able to include a comprehensive discussion of the results of liver therapy.

*Folklore of the Teeth**

DR. KANNER has made a very complete collection of popular superstitions and fancies, ancient, modern, and savage, on the subject of the teeth. Ignorance and superstition while often ludicrous are sometimes dreadful weapons for evil. The savage who destroys his child if the wrong tooth comes in first is pitiable.

We laugh with the author when he tells of the ancient astronomer devoting his life to counting the stars and finding upon questioning that he did not know the number of teeth in his own head. We are interested in the historical bits such as that Louis XIV born with teeth already erupted commenced life by making things disagreeable for the first person with whom he had direct contact, namely his wet-nurse. The story of St. Apollonia, the Patron Saint of Toothache, is interesting and especially so since the author traces the gradual evolution of the present miraculous story from the original bare facts.

The book is very pleasant reading.

Elementary Bacteriology†

IT IS a pleasant surprise to find in an elementary textbook a breaking away from the old traditional methods and the writing of a volume which makes a consecutive story from start to finish and is interesting reading even to one who has had more than the elementary experience with the subject. The first four chapters deal with generalizations, the history of bacteriology, the function of bacteria for good and for evil, and these are followed by chapters presenting in logical sequence morphology, classification, chemistry, food requirements, metabolic activity, influence of environment and interaction of bacteria with their environment, the carbon cycles and nitrogen cycles, nitrogen fixation, the phosphorus and sulphur cycles. Later come chapters on specific applications such as bacteriology of milk food poisoning, bacteria in the arts and industries, bacteria as the cause of disease, immunity, bacteriophage. Then come discussions of specific diseases with a final chapter on filtrable viruses. There is nothing new or startling in the book, in the sense of a new outlook or theory of bacteriology. If there were this would not be a textbook of elementary bacteriology. But the work recommends itself chiefly because it is easy and interesting reading and holds the attention throughout its pages.

Microchemistry‡

THIS is the fourth edition since 1921 of this well-known little manual of only 200 pages which has already been translated into Russian, Italian, and Spanish. No attempt is made to include all of the methods which have been published or used but only those are included which have been found satisfactory in the author's own laboratory. Methods are given, not only for the usual constituents of urine and blood but also for the common elements important in biochemistry and for oxygen and carbon dioxide in blood, including van Slyke's method for the alkaline reserve.

*Folklore of the Teeth. By Leo Kanner, M.D., Yankton State Hospital, Yankton, South Dakota. Cloth. Illustrated. 316 pages. The Macmillan Company, N. Y., 1928.

†Elementary Bacteriology. By Joseph E. Greaves, M.S., Ph.D., Professor of Bacteriology, Utah Agricultural College, Logan, and Ethelyn O. Greaves, M.S. Illustrated. Cloth. Pp. 506. W. B. Saunders Co., 1928.

‡Micromethods for the Quantitative Determination of the Constituents of Urine, Blood, and Organs in small Quantities for Clinical and Experimental Purposes. By Ludwig Pincusses. Fourth Edition. Published by Georg Thiene, Leipsig.

*The Advances in Microchemistry**

THIS is a much larger and more pretentious work than Pincussen's book as it is an attempt to cover all the various fields in which microchemistry has been found useful. Probably most of us have thought of microchemistry in connection with the remarkable analysis which can be made now from a small sample of blood but it has been developed also for use in both the qualitative and quantitative analyses of both organic and inorganic substances. It is remarkable how complete an analysis can be made from a little chip weighing a few milligrams or from a few borings of some precious heirloom or antiquarian relic. The author gives both the general and the specific methods of microchemistry in all of these fields.

The Spectrochemical Analysis of Natural Organic Coloring Matters†

THIS is really a second edition of a book published in 1909 under the title Clinical Spectroscopy by the same author. It considers the spectroscope and the spectroscopic methods useful in the study of the respiratory pigments chiefly. Most of the text is given to hemoglobin and all of its various forms and compounds and to its decomposition products including the pigments of the bile and urine. It also considers the closely related chlorophyl, other biologic pigments such as carotin, lutein, urorosein, and indigo, and the color producing cholesterol.

Die Methodik der Fermente‡

THIS is a work more or less coordinate with Oppenheimer's well-known work on Ferments and is designed to contain the practical parts and methods which could not be included in the original work.

Lieferung I contains chapters on polarimetry, refractometry, nephelometry, spectral-photometry, and colorimetry as well as chapters on viscosity, surface tension, hydrogen-ion concentration, electrical conductivity, and osmotic pressure.

Lieferung II contains the work on substrates and the methods for obtaining and purifying enzymes.

Erratum

Through an error the review of "The Treatment of Chronic Arthritis and Rheumatism" by H. Warren Crowe, the title of which appears on page 387 of the January, 1929, number, will be found on page 481 of the February number under the title "Chronic Rheumatic Diseases, Their Diagnosis and Treatment."

The review of "Chronic Rheumatic Diseases, Their Diagnosis and Treatment" by Thomson and Gordon, the title of which appears on page 481 of the February number, will be found on page 387 of the January number under the heading "The Treatment of Chronic Arthritis and Rheumatism."

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*The Advances in Microchemistry. By Gustav Klein and Robert Strebinger. Published by Franz Deuticke, Leipzig and Vienna, 1928.

†The Spectrochemical Analysis of Natural Organic Coloring Matters. By Prof. Otto Schumm. Pub. By Gustav. Fischer, Jena, 1927.

‡Die Methodik der Fermente (The Methods Used in the Investigation of Ferments). By Carl Oppenheimer and Ludwig Pincussen. Georg Thieme, Leipzig, 1927.

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EDITORIALS

The Transmission of Leprosy

ALTHOUGH there can be no manner of doubt that leprosy is an infectious disease and that as such it can only be acquired from an antecedent case, nothing accurate is known concerning the method or methods by which the disease is transferred or of how or where lepra bacilli enter the body."

With this succinct statement of an age-long problem Vedder[1] begins a very interesting report of an experimental study in which man was used.

While the reports of numerous observers have shown that nasal lesions are common, so common that the respiratory tract at one time was regarded as the common portal of the entry in leprosy, most authorities now believe that this is not so. The present opinion rests upon the observations that exclusive leprosy of the nose has never been reported, that positive nasal smears are not encountered in the absence of positive skin lesions, and that the frequency of the first observable lesions on the face together with the free communication with the lymph channels of the nose leads to early nasal infection.

The skin, then, appears to be the most common portal of entry of this disease and, in 75 per cent of cases, the skin of an exposed part of the body.

This being accepted, we are only on the threshold of the problem and the means whereby invasion occurs have yet to be determined beyond doubt.

Leprosy is regarded by almost all authorities as a contagious disease and from time immemorial popular opinion has always assigned contact as an important if not the only avenue of transmission.

The discharge of lepra bacilli in enormous numbers from ulcerating lesions and in various excreta and secretions, the fact that leprosy is often a familial disease, and the fact that the density of population influences the incidence of leprosy, all lend support to the importance of contact as a method of transmission.

While not denying that contact may be one method of transmission, there is nothing in the evidence, as Vedder points out, which indicates that it is the only, or even the most important avenue of infection.

As opposing evidence are the frequent observations of sharply circumscribed endemic foci; the fact that between infected husbands and wives, the uninfected partner contracts the disease in usually only from 5 to 6.5 per cent; that only about one person in twenty living in close contact with lepers contracts the disease; the freedom from leprosy of doctors, nurses, and attendants, in leprosaria; and the consistent failure, except in one disputed case, to inoculate man experimentally.

As Hirsch[2] who has exhaustively searched the literature in relation to contact infection says: " . . . there is not a single fact that positively and irrefutably shows the transmission of the disease through contagion; on the other hand, there are very cogent facts that contradict such a conception since they are in complete contradiction with all experience concerning the transmission of true contagious diseases."

Most convincing of all, are those cases, several of which have come under Vedder's own observation, in which the disease has occurred in Europeans or Americans none of whom were ever in intimate contact, and few of whom recall even casual contact with lepers.

In view of the evidence thus briefly outlined the possibility that leprosy might be transmitted by some intermediate agency such as the bites of insects very naturally arises and is referred to by numerous investigators. Vedder states that there are a number of considerations favoring this conception and quotes from the literature evidence indicating that bedbugs, lice, fleas, ticks, mosquitoes, and skin parasites *may* be vectors of leprosy.

In Vedder's opinion, if leprosy is conveyed by insects, one of the most important should be the mosquito, since cases occurring without known previous contact are best explained by a biting, flying insect.

He experimented, therefore, with Aedes aegypti, raised in the laboratory from the eggs.

The females thus raised were allowed to bite cases of macular or tubercular leprosy, the skin areas used being first examined microscopically to ensure the presence of numerous bacilli.

The mosquitoes were then killed and blood expressed from the abdomen examined. Undoubted lepra bacilli were found in 41 per cent. It appears, therefore, that under proper circumstances leprosy undoubtedly *may* be transmitted by the bite of mosquitoes.

Two human experiments are reported upon by Vedder the subjects being volunteers from the Bilibid prison, long term prisoners conforming to the following conditions:

1. He must freely volunteer, knowing the nature of the experiment.
2. He must be a long term prisoner.
3. He must be a young man in perfect health.
4. There must be no leprosy in the family nor any history of contact with lepers, and he must have been in isolation at least two years in Bilibid to exclude the development of leprosy from any other source than the experiment.

Three such volunteers were secured, one being used as a control and inoculated by an intradermal hypodermic injection, the second bitten on three different occasions by a total of 82 freshly infected mosquitoes, and the third bitten by 41 freshly infected mosquitoes allowed to feed repeatedly.

The experiments were conducted in 1927 and one year later none of the prisoners showed any evidence of leprosy, this being in the nature of a preliminary report.

Vedder believes that numerous similar experiments are not only desirable but, in view of the importance of the subject, justifiable.

REFERENCES

1. Vedder, E. B.: A Discussion of the Etiology of Leprosy, With Especial Reference to the Possibility of the Transference of Leprosy by Insects, and the Experimental Inoculation of Three Men, Philippine J. Sc. **37**: 3, 1928.
2. Hirsch: Handb. der Historisch-Geographischen Path. **2**: 33, 1883.

—*R. A. K.*

The Etiology of Trachoma

AMONG the last studies completed by the late Dr. Hideyo Noguchi, whose untimely death was a severe loss to experimental medicine, there was a series of investigations of trachoma the result of which is published as a complete and well illustrated report.*

While numerous bacteriologic studies have been made in this disease none, prior to this present investigation had found any microorganism satisfactorily or entirely fulfilling the postulates essential to its recognition as an etiologic agent.

Noguchi's experiments, which are reported in great detail, have resulted in the discovery of a new bacterium (Bacterium granulosis N.Sp.), which he believes to be the etiologic agent in trachoma. To support this belief strong confirmatory evidence is produced.

Bacterium granulosis is described as a small, motile gram-negative organism, somewhat resembling those of the xerosis group from which it is distinguished, however, by its biologic and chemical characteristics.

*Noguchi, H.: The Etiology of Trachoma, J. Exper. Med., Supplement 2, **48**: No. 2, 1, 1928.

When inoculated into chimpanzees, Macacus monkeys, and the baboon it produces lesions which grossly and histologically are characteristic of trachoma in the human being. The organism can be found in the lesions so produced, is found in sections from human trachomatous lesions, and is now in its fifth series in animals (culture to rhesus, rhesus to rhesus, rhesus to chimpanzee, chimpanzee to chimpanzee, and chimpanzee to rhesus).

The experimental chronic granular conjunctivitis produced by Bacterium granulosis preserved its clinical and histologic characters throughout several passages and the lesions have been shown to be infective as early as seventeen and as late as two hundred and four days after the original culture inoculation.

No other organism obtained from human cases of trachoma produces in animals comparable effects.

It appears, therefore, that the etiologic agent of trachoma has at last been isolated.

—*R. A. K.*

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News and Notes

The next annual meeting of the American Society of Clinical Pathologists will be held in Portland, Oregon, July 5, 6, and 8, 1929. All members are urged to make plans to attend this convention which besides promising a great intellectual feast will also enable them to visit our great far west.

The attention of the members of the American Society of Clinical Pathologists is called to the fact that at our next convention in Portland, Oregon, one-half day will be devoted to a group of presentation and general discussion of the subject of "Undulant Fever." Participation in this symposium by all the members is earnestly requested by the Program Committee and Research Committee.

Members will kindly report any change in address to the Secretary of the American Society of Clinical Pathologists, Metropolitan Building, Denver, Colorado.

Members who have not made reservations for the next convention should do so immediately by writing direct to Dr. H. H. Foskett, Medical Arts Building, Portland, Oregon. In view of the crowded condition of the Portland Hotel, our headquarters, during the American Medical Association Convention, members who have already made reservations for both the A. S. C. P. and the A. M. A. may be obliged to move to another hostelry during the A. M. A. convention. Accommodations in desirable hotels, however, are ample and Dr. Foskett will give all our Fellows his personal attention.